Listerellosis in Domestic Animals

A technical discussion of field and laboratory investigations

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Listerellosis in Domestic Animals

Robert Graham, N. D. Levine, and C. C. Morrill[1]

Listerellosis has only recently been recognized as a specific disease of domestic animals in Illinois. It has, however, been previously recognized in other states, as well as in foreign countries. The causative organism, *Listerella monocytogenes,* was first isolated by Murray, Webb, and Swann in England in 1926 during an epizootic among laboratory rabbits and guinea pigs. Pirie (1927) encountered a similar disease in a South African rodent, the gerbille (*Tatera lobengulae*), and named it "Tiger river disease."

Listerella was first recognized as a cause of disease in domestic animals by Gill (1931) when he isolated the organism from sheep in New Zealand and gave the name "circling disease" to the encephalitis which it caused. Since that time listerellosis has been reported in the goat, cow, pig, fox, chicken, rabbit, and man. To date the causative organism has been isolated from approximately nine different hosts in the United States and eight in other countries (Table 1).

The clinical symptoms and pathologic lesions associated with *Listerella* infection vary with the species of the host. In the rabbit, guinea pig, and gerbille a generalized infection associated with a circulating monocytosis and necrotic liver foci is characteristic. In the chicken the disease is also generalized and massive necrosis of the myocardium may occur. The single fox from which *Listerella* was isolated by Cromwell, Sweebe, and Camp (1939) suffered from a distemper-like disease. Jones (1940) reported isolation of a *Listerella*-like organism from the organs of 14 out of 27 cases of equine periodic ophthalmia, but their taxonomic relations to *Listerella* and their relation to the disease is uncertain. Later in an extensive discussion on periodic ophthalmia Jones (1942) failed to mention this organism.

In man *Listerella* is usually associated with meningitis or meningoencephalitis. Nyfeldt (1929, 1932) and Schmidt and Nyfeldt (1938) reported the isolation of *Listerella* from a number of cases of infectious mononucleosis, of which it was believed to be the cause. Pons and Julianelle (1939) obtained a true *Listerella* from a single case of infectious mononucleosis, but further study reported by Julianelle (1940A, 1941A) indicates that the association was probably fortuitous.

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TABLE 1.—REPORTS OF SPONTANEOUS LISTERELLOSIS

Host	Observer	Date report was published[a]	Place of origin or study	Type of disease
Rabbit	Goodpasture	1924	Tennessee	Encephalitis
	Murray, Webb, and Swann	1926	England	Generalized infection
	Paterson	1940C	England	Generalized infection
	Henricson	1943	Sweden	Generalized infection
Guinea pig	Murray, Webb, and Swann	1926	England	Generalized infection
Gerbille	Pirie	1927	South Africa	Generalized infection
Sheep	Gill	1931, '33, '37	New Zealand	Encephalitis
	Doyle[b]	1932	Indiana	Encephalitis
	Ten Broeck[c]	....	New Jersey	Encephalitis
	Jungherr	1937	Connecticut	Encephalitis
	Graham, Dunlap, and Brandly	1938	Illinois	Encephalitis
	Morin	1938	Illinois	Encephalitis
	Biester and Schwarte	1939	Iowa	Encephalitis
	Paterson	1939A, '40D	England	Abortion
	Graham, Hester, and Levine	1940B	Illinois	Encephalitis
	Olafson	1940	New York	Encephalitis
	Pallaske	1940	Germany	Encephalomyelitis
	Cross	1941	Colorado	Encephalitis
	Henderson	1941	Illinois	Encephalitis
	Hoffman	1941	California	Encephalitis
	Jensen and Gay	1941	Illinois	Encephalitis
	Muth and Morrill	1942	Oregon	Encephalitis
	Pomeroy, Fenstermacher, and Andberg	1943	Minnesota	Encephalitis
Goat	Olafson	1940	New York	Encephalitis
	King	1940	New Jersey	Encephalitis
	Gifford and Eveleth	1942	Arkansas	Encephalitis
Cattle	Matthews[b]	1928	Indiana	Encephalitis
	Jones and Little	1934	New Jersey	Encephalitis
	Fincher[b]	1935	New York	Encephalitis
	Graham, Dunlap, and Brandly	1938	Illinois	Encephalitis
	Graham, Hester, and Levine	1939, '40A	Illinois	Abortion
	Graham, Hester, and Levine	1940B	Illinois	Encephalitis
	Biester and Schwarte	1941B	Iowa	Encephalitis
	Paterson	1941	England	Hepatitis
	Schwarte and Biester	1942	Iowa	Encephalitis
Pig	Evans and Sawyer	1942	Vermont	Abortion
	Pomeroy, Fenstermacher, and Andberg	1943	Minnesota	Encephalitis
	Slabospits'kii	1938	Russia	Pox-like
	Biester and Schwarte	1940, '41A	Iowa	Encephalitis
Horse	Jones[d]	1940	Virginia	Periodic ophthalmia
Fox	Cromwell, Sweebe, and Camp	1939	Illinois	Distemper-like
Man	Atkinson[e]	1917	Australia	Meningitis
	Dumont and Cotoni	1921	France	Meningitis
	Baldridge, Rohner, & Hausmann[e]	1926	United States	Infect. mononucleosis
	Nyfeldt	1929, '32	Denmark	Infect. mononucleosis
	Tesdal[e]	1934	Norway	Meningitis
	Schultz, Terry, Brice, and Gebhardt	1934, '38	California	Meningitis
	Burn	1934, '35, '36	Connecticut	Meningitis
	Gibson	1935	Scotland	Meningitis
	Allen[f]	....	Connecticut	Meningitis
	Carey	1936	Massachusetts	Meningitis
	Poston, Upchurch, and Booth	1937	North Carolina	Meningitis
	Schmidt and Nyfeldt	1938	Denmark	Infect. mononucleosis
	Cislaghi[g]	1938	Italy	Meningitis
	Pons and Julianelle	1939	Missouri	Infect. mononucleosis
	Porzecanski and de Baygorria	1939	Uruguay	Meningitis and otitis media
	Wright and Macgregor	1939	Scotland	Meningitis
	Wagner and Porter[h]	....	Iowa	Meningitis
	Savino	1940A, B	Argentina	Meningo-encephalitis
	Fischer	1941	Uruguay	Meningo-encephalitis
Chicken	Ten Broeck[c]	....	New Jersey	Generalized infection
	Paterson	1937, '39A	England	Generalized infection
	Watkins[i]	....	England	Generalized infection
	Pallaske	1940	Germany	Generalized infection
	Cole	1941	New York	Generalized infection
	Hurt, Levine, and Graham	1941	Illinois	Generalized infection

[a]These reports are listed in the bibliography. [b]Did not identify as listerellosis but it probably was this disease. [c]Observation reported by Seastone (1935). [d]Relation of this strain of *Listerella* to that isolated by other investigators is not certain. [e]Classified organism as a diphtheroid, but it may have been *Listerella*. [f]Observation reported by Burn (1936). [g]Did not isolate *Listerella*. [h]Observation reported by Porter and Hale (1939). [i]Observation reported by Paterson (1939).

Indeed, more recent work, such as that of Nettleship (1942), suggests that infectious mononucleosis in man is caused by a filtrable virus.

Listerellosis in ruminants is usually manifested by an encephalitis or encephalomyelitis. Meningitis may also be present. Abortion not accompanied by clinical symptoms of encephalitis has been reported in the cow by Graham, Hester, and Levine (1939, 1940A) and by Evans and Sawyer (1942) and in sheep by Paterson (1939A, 1940D).

Because there was little information on listerellosis when the first recognized outbreaks occurred in Illinois, the Station published detailed descriptions of these outbreaks in order to assist veterinarians in the clinical diagnosis of the disease. When listerellosis was recognized more frequently, experimental studies were begun on the bacteriology, immunology, epizoology, hematology, and histopathology of the disease. This bulletin brings together the information obtained in these studies at the Illinois Station and summarizes the findings of other investigators.

FIELD OUTBREAKS IN ILLINOIS

Sheep

Listerellosis has been found in several flocks of Illinois sheep. Three of the outbreaks have been described by Graham, Dunlap, and Brandly (1938), and Graham, Hester, and Levine (1940B). Henderson (1941) reported on the reappearance of the disease in one of these flocks.

First outbreak. The first outbreak of listerellosis which was recognized in Illinois occurred in a flock of 300 western feeder lambs during the winter of 1937-38. The lambs had been bought on the market on November 1. They probably included animals from several sources, and there may have been some native lambs in the group. Losses began in December, about 6 weeks after the lambs had been purchased, and continued into February. About 30 lambs, or 10 percent of the flock, died during this period. Death usually occurred about 4 days after the symptoms were first observed. Early in the outbreak, all affected animals died, but later on some lambs recovered.

ORIGIN OF NAME OF ORGANISM—When Murray, Webb, and Swann first isolated the organism in 1926 they gave it the name *Bacterium monocytogenes* because the disease was characterized by an increase in the number of circulating monocytes. Recognizing the causative organism of "Tiger river disease" to be a new genus, Pirie (1927) gave it the name *Listerella* and suggested the specific name *hepatolytica* because of the liver lesions it produced. When an exchange of cultures showed that the organisms isolated by Pirie and by the workers in England were the same, Pirie withdrew the name *hepatolytica* in favor of the earlier name *monocytogenes*. After Becker (1939) pointed out that the generic name *Listerella* had already been used for a mycetozoon, Pirie (1940) suggested that a new name, *Listeria,* be used. This name was not adopted, however, because it had already been given to a certain plant group.

The lambs were in good condition prior to the appearance of the disease, and were receiving an adequate diet of corn silage, chopped corn fodder, oats, corn, and oat hay. Altho the silage was of good quality, it was thought at first that it might be a factor in causing the disease, and the owner ceased feeding it early in the outbreak, substituting a good grade of alfalfa. However, the change had no visible effect on the course of the disease.

According to the owner, the affected lambs first displayed symptoms of dullness, their ears drooped, and their eyes appeared dull. Signs of impaired vision followed, and the animals walked into objects or walked close to them before observing them. On about the third day after the symptoms were first observed, the sheep began to walk in circles either to the right or left and to push or lean against solid objects. Conjunctivitis and opacity of the cornea were noted in some cases. A convulsive seizure was noted in one lamb, and others stood with saliva drooling from the mouth.

Six lambs were received for autopsy at the Animal Pathology and Hygiene laboratory, University of Illinois, during the course of the outbreak. Two lambs were in the early stages of the disease. Sluggishness, dull eyes, and drooping ears were observed. No gross pathologic lesions were found at autopsy. The urine was negative to sugars and ketones. Tallquist blood hemoglobin levels were 85 percent and 90 percent. Serum calcium and inorganic phosphorus were normal, but both animals were hyperglycemic. A definite leucopenia was present in both lambs, total leucocyte counts being 3,950 and 4,050.

A Gram-positive rod with the morphologic characteristics of *Listerella* was isolated from the medulla oblongata (hereinafter designated simply as the medulla) of each lamb on plain or liver agar plates and thru meat mash. Practically pure cultures developed on the plates after 2 days' incubation at 37° C. A saline suspension of the organism produced conjunctivitis and keratitis when dropped into the eyes of guinea pigs (Fig. 1).

A rabbit, a guinea pig, and a pigeon were inoculated subcutaneously with a saline suspension of a composite sample of the medullae of the two lambs, but these animals were released healthy after a month's observation. A rabbit, a guinea pig, and a pigeon were inoculated subcutaneously with a saline suspension of a washed agar culture of the organism recovered from the medulla. The rabbit died after 18 days, but cultures of heart blood, brain, and bone marrow were negative. The guinea pig and pigeon remained healthy.

Three of the lambs were held at the laboratory for observation for 4 days. One was apparently a cull and exhibited emaciation, weakness, and conjunctivitis, altho its temperature and blood picture were normal. Upon autopsy, atelectasis of the cardiac lobes of the lungs was observed, numerous *Cysticercus tenuicollis* were present in the pan-

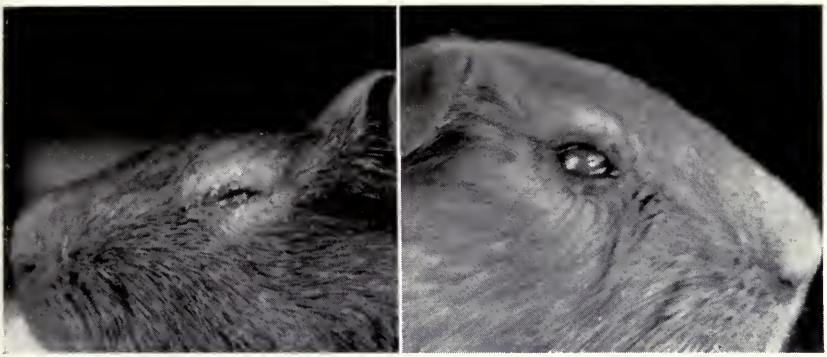

FIG. 1.—GUINEA PIGS AFTER SUPRACONJUNCTIVAL EXPOSURE TO LISTERELLA
Both guinea pigs have conjunctivitis; the one to the right has keratitis also.

creatic region, and there appeared to be an excessive amount of fluid in the spinal canal. However, *Listerella* was not isolated from cultures of the cerebrum, medulla, lumbar cord, or heart blood, and a rabbit, 2 guinea pigs, and 2 chickens inoculated subcutaneously with a saline suspension of the medulla remained healthy.

The other 2 lambs, which had been in good flesh, became progressively weaker and then finally comatose. Progressive torticollis was observed. The lambs' temperatures were from 1 to 3 degrees above normal from the time of admission until they were destroyed. Total erythrocyte and leucocyte counts were within the normal range, but 1 lamb exhibited a relatively high percentage of neutrophiles (84 percent neutrophiles, 8 percent lymphocytes, and 8 percent monocytes). The other lamb presented a normal differential count. Blood sugars were within the normal range.

Upon autopsy of a lamb which exhibited a mucopurulent nasal discharge and conjunctivitis, no gross lesions were found in the visceral organs, but a relatively large amount of pericardial fluid was found and the bones were relatively soft. An excess of spinal and cranial fluids was present, but the brain appeared to be grossly normal. *Listerella* was isolated on plain and blood agar from the lumbar cord and medulla (Fig. 2), but not from the peritoneal fluid, pericardial fluid, heart blood, liver, cerebral cortex, or hippocampus. An emulsion of the medulla in physiological salt solution was inoculated subcutaneously into 2 guinea pigs, 2 rabbits, and 2 pigeons. Four days later one guinea pig was sick and the other dead. *Listerella* was isolated by direct cultural methods from the brain of the sick guinea pig and from the heart blood of the dead guinea pig. *Listerella* was also isolated from the bone marrow of a rabbit which died 15 days after inoculation. The other rabbit and the 2 pigeons remained healthy.

No gross lesions were noted on autopsy of the third lamb of this group. Bacteriologic cultures were made of the same organs as for the previous lamb, but *Listerella* was isolated only from the medulla and on blood agar. A saline suspension of the medulla was inoculated subcutaneously into 2 rabbits, 2 guinea pigs, and 2 pigeons; all remained healthy.

FIG. 2.—NATURAL LISTERELLOSIS IN SHEEP

The culture of *Listerella* from the sheep medulla (*upper left*) was incubated 48 hours at 37° C. The large white masses are medulla tissue. The eye of the sheep shown below to the right is affected with keratitis; the other is normal.

On February 6 a sixth lamb was received from the same flock. It was comatose and had been unable to stand for 5 days. Conjunctivitis and keratitis of the left eye were present (Fig. 2). At autopsy the subdural space was found to contain a slightly excessive amount of fluid, and a congested area was observed in the left lung, but no other gross lesions were present. Cultures of the heart blood, liver, pericardial fluid, cerebral cortex, medulla, lumbar cord, and hippocampus were made on plain agar, liver agar, and meat mash, but *Listerella* was isolated only from the medulla on plain agar. Two guinea pigs, 2 rabbits, 2 chickens, and 2 pigeons were inoculated subcutaneously with a saline suspension of the medulla. One guinea pig died 6 days later, and *Listerella* was recovered from the cerebrum and heart blood. The rabbits died 3 and 16 days after inoculation, but bacteriologic cultures were negative to *Listerella.* The chickens, pigeons, and second guinea pig remained healthy.

Three attempts were made to determine whether a virus was present in the brains of the affected sheep in this flock. A Berkefeld-N filtrate, made of the brain tissue of a lamb from which *Listerella* was isolated, was cultured to establish freedom from bacteria and then a rabbit, a guinea pig, and 2 chickens were inoculated intracerebrally with it. One chicken died 13 days later, and cultures made from its organs were negative. The other animals remained healthy. A Berkefeld-N filtrate from a second lamb was cultured for bacterial sterility and inoculated intracerebrally into a rabbit, a guinea pig, and 2 chickens. The guinea pig and 1 chicken died a week later, but the other animals remained healthy. Death of the chicken was probably due to trauma. Bacteriologic cultures of the organs were negative in both cases. An emulsion was made of the brains of the guinea pig and chicken in physiological salt solution and inoculated intracerebrally into 3 chicks and intravenously into 1 chick. All four birds remained healthy. The same suspension was inoculated onto the chorio-allantoic membranes of a dozen 12-day-old chick embryos. No lesion or other evidence of any ill effect was observed.

A composite suspension made of the medullae of 3 other lambs from this flock was filtered thru a Berkefeld-N candle, tested for bacterial sterility, and inoculated intracerebrally into 4 young chickens and 2 guinea pigs. One guinea pig, which exhibited nasal hemorrhage and a tendency to turn to the right immediately after inoculation, died 12 days later. Bacteriologic cultures were negative, and death was probably caused by injury at the time of inoculation. All the other animals remained healthy. The chorio-allantoic membranes of 12-day-old chick embryos were inoculated with the same filtrate without visible effect.

Second outbreak. This outbreak occurred in a flock of 100 ewes in central Illinois. Illness and slight losses had occurred over a period of about a month when, in March, 1938, a ewe was brought to the

Animal Pathology and Hygiene laboratory for diagnosis. She was the eighth animal in the flock to be affected. Six other ewes had died, but a yearling lamb which had been affected was recovering altho still exhibiting some locomotor incoordination.

No relation between the feeding program and the disease was suggested. Illness was observed for about 5 days before death. The principal symptom was a tendency to walk in a large circle. The ewe presented for diagnosis exhibited neither keratitis nor conjunctivitis, altho a dried discharge was present on the left frontal area of the head and there was a watery nasal discharge. The animal circled to the left when walking and often leaned with her left side against objects or stood in a corner.

Upon autopsy the thoracic and abdominal viscera appeared normal except for a heavy *Oesophagostomum* infestation. The meninges over the cerebrum appeared slightly rough by reflected light, and an increased amount of cerebrospinal fluid was present. Blood hemoglobin was 80 percent (Tallquist method), the total erythrocyte count 7,200,000 per cu. mm., and the total leucocyte count 14,300 per cu. mm. A differential leucocyte count revealed 65 percent neutrophiles, 32 percent lymphocytes, and 3 percent monocytes.

The heart blood, spleen, liver, cerebrum, medulla, lumbar cord and hippocampus were cultured on liver agar plates and meat mash tubes. *Listerella* was recovered only from the medulla.

Third outbreak. Losses from listerellosis in a flock of 800 ewes in southern Illinois started during the winter of 1938-39. About 40 animals had died when, in the latter part of April, 1939, 2 were brought to the Animal Pathology and Hygiene laboratory for diagnosis. The symptoms which were observed in the flock were similar to those in the first and second outbreaks. At autopsy no gross lesions were found in either sheep. The medulla of one was cultured on plain and liver agar plates and in meat mash and brain mash. *Listerella* was recovered only from the plain and liver agar plates. The medulla of the second ewe was cultured on plain agar plates and in meat mash, brain mash, 10-percent serum broth, and Rosenow's semisolid brain agar. *Listerella* was recovered from the meat mash and serum broth.

A rabbit and 2 chickens were inoculated intravenously and a guinea pig was inoculated intraperitoneally with a saline suspension of the *Listerella* culture. The rabbit died 2 days and the guinea pig 5 days after inoculation. *Listerella* was recovered from the heart blood and brain of both. The chickens remained healthy.

A third ewe from the same flock was later submitted to the laboratory for diagnosis. It was one of 50 which had been transferred to a farm in central Illinois. No symptoms had been observed in this group or in the other sheep on the farm until 18 days later, when this animal was found in the pasture unable to rise.

No gross lesions were observed upon autopsy, altho a moderate parasite infestation was present. The cerebrum and medulla were cultured on plain agar plates and in meat mash, serum broth, and Rosenow's semisolid agar; *Listerella* was isolated from both organs in all media. This case is of particular interest both because the organism was isolated from the cerebrum and because it was clear that the incubation period was at least 18 days, the time which had elapsed since the ewe was in the flock where she undoubtedly had been exposed.

The sheep on the farm in southern Illinois where the ewe was exposed were observed carefully thruout the three succeeding years. No losses attributable to listerellosis occurred during the winter of 1939-40, but the disease reappeared in December of the next year. This recurrence has been described by Henderson (1941).

At the time the disease reappeared the flock was in winter quarters. It consisted of 34 native purebred rams and 860 western ewes, the latter including a few purebreds. It was divided into three groups of 240 ewes each and four smaller groups.

Four ewes in the flock died during the last 10 days of December, 1940, and the losses continued into March, 1941. The symptoms were similar to those already described. One of the first symptoms was refusal to come to the racks at feeding time. Altho not all affected animals showed this reluctance to feed, the symptom was found helpful in detecting early cases. Also, very early in the disease the animals tended to walk sideways. Most of the affected animals walked in a circle to one side or the other, and even those that did not walk in circles tended to hold their heads to one side in the terminal stages of the disease. Affected animals were frequently unable to rise but performed running movements while lying on their sides. Distinct conjunctivitis was not often observed in this flock. The period between first observation of symptoms and death varied from 1 to 4 days. Two typically affected ewes were brought to the laboratory for diagnosis. Mild congestion of the meninges was the only gross lesion observed at autopsy. Bacteriologic cultures of the medullae from both animals revealed *Listerella*.

On December 30, 1940, each animal in the flock had been inoculated subcutaneously with 2 cc. of a formalin-killed broth culture of *Listerella*. On January 22, 1941, a 3-cc. dose of the same bacterin was similarly administered. During the first 2 weeks in March half the ewes were given 5 cc. and the other half 10 cc. of the bacterin subcutaneously, and the lambs each received an injection of 2 cc. and then one of 3 cc. after 2 weeks.

During January, 1941, 26 ewes and 3 lambs died of listerellosis. The disease appeared to be subsiding in February, since only 2 animals died until the last 5 days of the month, when 5 more ewes died. During March 12 ewes and 10 lambs succumbed.

In April the flock was turned onto pasture, and the losses ceased. A total of 55 ewes, 2 rams, and 19 lambs had died during the course of the outbreak.

Since Porter and Hale (1939) had reported that sulfanilamide gave favorable results in the treatment of mice experimentally infected with *Listerella,* and since Olafson (1940) had suggested its use in the treatment of sheep, an attempt was made to cure affected sheep in this flock by the use of sulfanilamide. The drug was administered to 27 ewes upon appearance of symptoms. It was given by mouth twice daily in doses of 60 to 180 grains until death or improvement occurred. Only 3 of the animals recovered and these had received the heavier dosage. None of these 3 animals was, however, quite typical clinically, and it is possible that their condition was not listerellosis.

Cultures were made of the medulla of one typically affected ewe which had been treated with sulfanilamide, but *Listerella* was not isolated. Failure to find the organism may have been due to the treatment. If this was the case, it is possible that the sulfanilamide was ineffective not because it failed to destroy the bacteria but rather because the brain had been irreparably damaged before it was used.

The disease reappeared in this flock during the winter of 1941-42, and its presence was confirmed by bacteriologic examination. The flock at this time contained 1,025 ewes. Experimental vaccination using large doses of bacterin had been carried out and may have had some influence upon reducing the number of cases. Nevertheless, a total of 46 ewes and 31 spring lambs died. The details of the vaccination experiment are given on page 84.

In August, 1941, 29 ewes and 17 lambs were transferred from this southern Illinois farm to another farm in east-central Illinois, where more than 400 sheep were kept. During the following winter and spring about 100 ewes and their lambs were kept in pens in a barn, each pen containing 10 ewes and their lambs. The sheep which had been brought from southern Illinois were distributed in various pens. Early in May, one of these transferred Southdown ewes became ill and died with symptoms typical of listerellosis. Bacteriologic examination, however, failed to reveal *Listerella.* A few days later a Hampshire ewe in another pen became ill, and *Listerella* was isolated from the medulla. Two of the spring lambs in the same pen also died, and *Listerella* was recovered from their medullae. The Hampshire ewe that died had been purchased in August, 1940, from another flock where an outbreak of the disease was later observed in April, 1941 (fifth outbreak, page 13).

On this farm in east-central Illinois no treatment was administered to any of the sick sheep or to the healthy ones in the same pens. Neither was a change made in the feeding and management practices, which were considered good. No further illnesses or deaths attributable to listerellosis occurred.

Fourth outbreak. During the winter and early spring of 1939-40 approximately 12 sheep died in a flock of 180 in western Illinois. Three lambs had died previously. According to the owner, the affected lambs seemed to "go crazy" and wandered around with a staggering gait. The ewes behaved similarly. The flock had been receiving sorgo silage, bean hay, and corn. After the local veterinarian suggested that the sheep might be getting too much carbohydrate, the amounts of corn and silage were decreased and alfalfa hay was added to the ration, but no appreciable effect on the losses was observed.

During the first week of April 1940, 2 ewes were delivered to the Animal Pathology and Hygiene laboratory for diagnosis. One had been found sick the day before; the other died en route. At autopsy corneal opacity was observed in both ewes, but no other gross lesions attributable to listerellosis were noted. One ewe had a moderately heavy stomach-worm infestation, but the other was in high condition and did not have an appreciable number of these parasites.

Bacteriologic cultures of the medullae of both ewes were made on plain agar plates and in meat mash. *Listerella* was isolated thru both media from one animal but not from the other.

A formalin-killed *Listerella* bacterin was sent to the local veterinarian for the remainder of the flock. Following the use of bacterin in doses of 2 to 5 cc., the disease disappeared. However, it is uncertain whether the bacterin was responsible.

Fifth outbreak. During the first week in April, 1941, 2 ewes were received for examination from a flock of 300 purebred sheep in northern Illinois. The flock was composed primarily of Shropshires and Oxfords, with smaller numbers of Hampshires, Suffolks, and Southdowns. At the time the ewes were received for examination, 4 or 5 lambs, a ram, and several ewes had died. The sheep had been fed alfalfa hay, oats, bran, oilmeal, silage, a mineral mixture, salt, and fresh water.

The symptoms in this flock have been described by Jensen and Gay (1941). They included circling in one direction or the other, torticollis, and occasional rapid twitching of the skin ("fly-shaker movement"). If the sheep survived for a few days, conjunctivitis and keratitis often appeared. Occasionally a mucous nasal discharge was present. Quite frequently one ear drooped, and Jensen and Gay noted pharyngeal paralysis in the later stages of the disease. They found no high temperatures in visibly affected animals, but noted that 2 ewes which were a little sluggish and had temperatures of 105° and 104.1° F. later exhibited symptoms of listerellosis. Hence they suggested that a fever might occur in a very early stage of the disease and be of assistance in recognizing it.

About two-thirds of the ewes in the flock were nursing lambs, and the disease occurred in both ewes and lambs. The course of the disease

was more rapid in the lambs; they died a few hours after symptoms were observed. The ewes usually lived 1 or 2 days after the appearance of symptoms, becoming prostrate and falling into a coma before death. Both the ewes and lambs commonly made running movements while lying on their sides, often causing the wool to come off and decubital sores to appear on the shoulder and hip next to the ground.

One ewe was dead on arrival at the laboratory. The other had a temperature of 102.4° F. and a total leucocyte count of 10,000 per cu. mm. At autopsy effusions varying in nature from serous to serofibrinous were found in the pericardial cavities of both animals. A few nodules caused by *Oesophagostomum* were present in the intestines, and the dead sheep exhibited a mild stomach-worm infestation. The spleen of the animal submitted alive seemed slightly swollen, and a few small kidney stones were present. Several *Oestrus ovis* larvae were found in the nasal passages of the live sheep but none in the dead one. The extremities of both animals were bruised to some extent as a result of continued forced movements.

Smears made from the hippocampi were negative to Negri bodies. The medullae were cultured on plain agar and meat mash, as were the heart blood, liver, and spleen of the freshly killed animal. *Listerella* was not isolated from either ewe on any of the media. Two rabbits and a guinea pig were inoculated subcutaneously with a saline suspension of the medullae, and all 3 died within a week. *Pasteurella* was isolated on bacteriologic examination from the heart blood of both the rabbits and the guinea pig. Had this organism been recovered from only one experimental animal, it would be considered doubtful whether it had originated in the sheep. However, since it was found in all 3, and since all the other animals in the laboratory colony remained healthy, it seems probable that *Pasteurella* was actually present in the sheep, altho perhaps only as a secondary invader.

Two more sheep were delivered to the laboratory from the same flock. One had received 180 grains of sulfanilamide by mouth the day before, and both were given 180 grain doses of sulfanilamide on the morning and evening of the day they arrived. One died that same evening, and the other 2 days later. *Listerella* was isolated from the medullae of both animals on plain and blood agar and in meat mash.

Losses in this herd were heavier than in any outbreak of listerellosis which came to the authors' attention. Most of the sheep were kept in a large barn where losses in the north wing were heavier than in the south wing. Very few deaths occurred among 80 yearlings kept in another barn with a number of cows. During the course of the outbreak, a total of 101 sheep and 3 goats died of listerellosis.

Two affected ewes in this herd were isolated in a small pasture and recovered without treatment. These were exceptional, however, since most affected sheep died.

When the attendant veterinarians were first called to see the flock, they made a tentative diagnosis of forage poisoning (Jensen and Gay, 1941). They reported that treatment with sodium thiosulfate both intravenously and orally was given 3 affected sheep, and Theradye solution (6 percent calcium borogluconate and .15 grain methylene blue per cc.) was given intravenously to 3 other sheep. All 6 animals died. Three affected sheep were given three daily doses of 30 to 40 cc. of 5-percent neoprontosil intravenously at the same time that they received three daily doses of 180 to 360 grains of sulfanilamide by mouth. These animals also died. However, 2 other ewes similarly treated very early in the disease recovered. Three sheep were treated with sodium iodide intravenously and potassium iodide by mouth, but this treatment was ineffective.

In cooperation with the local veterinarians 50 ewes and 5 lambs in the north wing of the barn were vaccinated with an experimental *Listerella* bacterin prepared by a commercial laboratory. Fifteen of these animals received three 1-cc. doses intradermally, and the other 40 received three 10-cc. doses subcutaneously. Seventeen of the rams in the west wing of the barn were similarly treated with a bacterin prepared by the Animal Pathology and Hygiene laboratory. The 75 head in the south wing of the barn, the 13 remaining rams, and the yearlings in the other barn were not vaccinated. Until July, losses continued to occur among both the vaccinated and unvaccinated sheep, and no significant difference in losses could be noted between the two groups. Hence it was felt that vaccination carried out as described above was of no value in this herd.

A controlled vaccination experiment was initiated in this herd in October, 1941, with a view to ascertaining whether vaccination would significantly affect the incidence of listerellosis the following winter. However, since the disease failed to reappear even in the control animals, no conclusions could be drawn.

Sixth outbreak. During the first week in June, 1941, a Horned Dorset ewe showing clinical symptoms of encephalitis was delivered to the laboratory from a farm in northern Illinois close to the premises on which the fifth outbreak of listerellosis had occurred. The ewe was brought in because the owner suspected listerellosis. No information was available on the size of the flock or the extent of the losses. At autopsy, no gross lesions were encountered except that the kidneys may have been slightly swollen. Very few stomach worms were present. The urine was strongly positive to sugar and contained a slight amount of albumin but was negative to bile, blood, and ketones. The medulla and liver were cultured on plain agar and meat mash, and *Listerella* was isolated from the medulla.

Seventh outbreak. On January 23, 1942, a live sheep displaying encephalitic symptoms was delivered to the laboratory from a flock in

north-central Illinois. Losses had started in this flock early in the month, and 10 or 11 animals had died when this specimen was submitted. According to the owner, symptoms included loss of muscular control and drooling. The sheep remained in a recumbent position, and a discharge from the nostrils was noted in some animals. The animals had been receiving hay, corn, oats, and a relatively large amount of silage when the disease appeared. The owner suspected poisoning and stopped feeding the silage, but this had no effect on the course of the outbreak.

The blood sugar was 150.29 mg. per 100 cc., a high value. The urine was strongly positive for sugar but ketones were absent.

The medulla was cultured on plain agar and blood agar plates, and *Listerella* was isolated on both media.

Summary of death losses among sheep. The losses due to listerellosis in the outbreaks among sheep are summarized in Table 2. From 4.5 percent to 33.5 percent of the sheep in different flocks died of the disease. The average death loss in the 5 flocks for which information was available was 10.6 percent, but if this figure is weighted to allow

TABLE 2.—LOSSES FROM LISTERELLOSIS IN OUTBREAKS AMONG SHEEP IN ILLINOIS

Outbreak No.	Animals in herd	Animals dead		Outbreak No.	Animals in herd	Animals dead	
		Number	Percent			Number	Percent
1	300	30	10.0	4	180	12	6.7
2	100	8	8.0	5	300	101	33.5
3 (1st year)	800	40	5.0	6	....	...	....
(3d year)	894	57[a]	6.4	7	....	12-15	....
(4th year)	1,025	46	4.5				

[a]Exclusive of 31 lambs born during the outbreak.

for the different number of sheep in each flock, the average death loss becomes 8.2 percent. The morbidity due to listerellosis is relatively low, but the mortality in affected animals is very high. In fact, recoveries are exceptional.

The locations of the outbreaks of ovine listerellosis recognized in Illinois are shown in Fig. 9. The disease is state-wide in distribution.

Cattle

Listerellosis has been recognized in eight herds of cattle in Illinois. Four of the outbreaks have been described by Graham, Dunlap, and Brandly (1938), and Graham, Hester, and Levine (1939, 1940A, 1940B).

First outbreak. During the middle of February, 1938, the heads

of 2 two-year-old steers were brought to the Animal Pathology and Hygiene laboratory for diagnosis. These steers had been part of a herd of 68 feeder steers on a farm in central Illinois. The animals had been purchased in the fall, but their origin was unknown. The herd was receiving Sudan grass, hay, good silage from a trench silo, ground corn, and soybean oilmeal.

Three steers in this herd had been lost from shipping fever, according to the owner, shortly after they arrived on the farm. Three other animals, including the 2 whose heads were brought to the laboratory, had died recently. Rabies was suspected.

The 2 steers had been sick for more than 2 weeks before they died. According to the owner, the first symptom was a staggering gait; the animals were gaunt, drooled saliva, and went down. They were unable to eat, but they drank altho slowly. During the first few days the head was held high, the animals looking toward the sky. The steers were in a coma for the last 4 days before death. The veterinarian who was called to attend them reported that their attitude suggested founder. The saliva was stringy, and there was an apparent paralysis of the muscles used for mastication.

Examination of the heads revealed an excessive amount of cerebral fluid, and in addition a purulent sinusitis was present in one animal. Negri bodies were not found. The medullae were cultured on liver and blood agar plates, and *Listerella* and *Pasteurella* were isolated from both.

After these steers became ill, the owner sent 36 head to market, and no other animals sickened.

Second outbreak. Early in May, 1939, an aborted 7-months bovine fetus was delivered to the laboratory for diagnosis from a herd of dairy cattle in northern Illinois. The herd was evidently free from brucellosis and had been tested only a month before with negative results. Microscopic examination for *Trichomonas foetus* was negative. The stomach contents of the fetus were planted on liver agar plates which were incubated aerobically and in an atmosphere of 10 percent carbon dioxide at 37° C. for 2 days. *Listerella* was obtained in pure and abundant culture, but *Brucella* could not be isolated. A rabbit which was inoculated subcutaneously with .5 cc. of the fetus's stomach contents died 10 days later, and *Listerella* was isolated from its heart blood. Unfortunately no studies could be made upon the cow that had aborted; her owner sent her to market immediately.

A rabbit was inoculated intravenously and a guinea pig intraperitoneally with a saline suspension of the organism isolated from the fetus. The rabbit died 2 days later and the guinea pig 4 days later. *Listerella* was recovered from the heart blood and brain of both animals upon bacteriologic examination. Two chickens inoculated intravenously with a saline suspension of the organism remained

healthy. Intravenous inoculation of other chickens, however, was followed by death. At autopsy of the sick chickens massive or focal necrosis of the myocardium was observed (Fig. 3) and *Listerella* was recovered from the heart blood. Two rabbits and 2 guinea pigs were inoculated supraconjunctivally with a saline suspension of the organ-

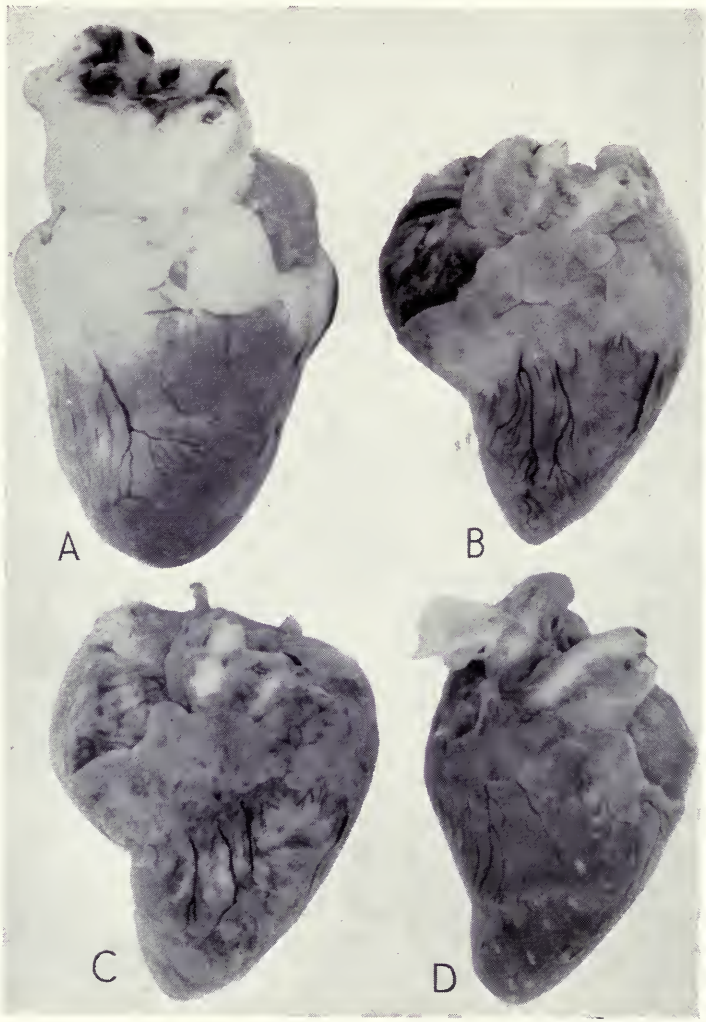

FIG. 3.—MASSIVE AND FOCAL NECROSIS OF THE MYOCARDIUM IN CHICKENS

Hearts **B** and **C** show massive necrosis and Heart **D** focal necrosis in chickens that died following intravenous inoculation with *Listerella.* Heart **A** is normal.

ism, with the result that conjunctivitis and corneal opacity developed in the guinea pigs and one of the rabbits (Fig. 4).

Since at that time no record of the isolation of *Listerella* from aborted mammalian fetuses had been published, a study was carried out to ascertain whether this *Listerella* strain might be responsible for the abortion.

A heifer 5 months in calf was purchased from a brucellosis-free herd. A test of the blood for *Brucella* agglutinins was negative. The heifer was inoculated intravenously with 3 cc. of a saline suspension of the *Listerella* strain (density McFarland Nephelometer Tube 10) from the aborted fetus. Her temperature rose to 104.2° F. on the

FIG. 4.—CONJUNCTIVITIS IN RABBIT AND GUINEA PIG

The rabbit (*upper*) and the guinea pig (*lower*) were each given a supraconjunctival inoculation with *Listerella* Strain 27681.

afternoon of the day of inoculation and was 105.6° F. on the following day. It then subsided to 102.8° F. during the next 5 days, after which it gradually rose to 104° F. on the tenth day after inoculation. On this day the heifer aborted and her temperature returned to normal.

After the heifer had been inoculated with the suspension of the *Listerella* she breathed rapidly and refused food for 4 days. Very little food was eaten until after the abortion, altho the animal drank a moderate amount of water. Loss of weight was evident during the period between inoculation and abortion. After this the heifer regained her appetite and made an uneventful recovery.

The blood picture was studied from the time of inoculation until 2 months after abortion. The total erythrocyte count did not change significantly during this period. The total leucocyte count was 13,000 per cu. mm. at the time of inoculation. It decreased to 6,450 three days later and to 5,000 on the day of abortion; in the course of the next 3 weeks it rose slowly to 11,000. A differential count at the time of exposure demonstrated 44 percent neutrophiles, 48 percent lymphocytes, 5 percent monocytes, and 3 percent eosinophiles. A marked change was present at the time of abortion, when 14 percent neutrophiles, 72 percent lymphocytes, 13 percent monocytes, and 1 percent eosinophiles were present. This represented an increase in percentage of lymphocytes and monocytes and a decrease in percentage of neutrophiles and eosinophiles. However, if these percentages are correlated with the total cell counts, it is seen that none of the cells actually increased in numbers. At the time of inoculation there were 5,720 neutrophiles per cu. mm., 6,240 lymphocytes, and 650 monocytes; when the heifer aborted, the neutrophiles had decreased to 700 and the lymphocytes to 3,600, the number of monocytes remaining constant at 650. Thus the change in the blood from the time of exposure to abortion was characterized by a marked decrease in neutrophiles and a smaller decrease in lymphocytes; the monocytosis was only relative and was of short duration, 3 percent monocytes being found 10 days after abortion. The neutrophiles and lymphocytes increased slowly, returning to the pretreatment level 5 weeks after abortion.

Agglutination tests for brucellosis were carried out on the blood of the heifer before inoculation, during her illness, immediately after the abortion, and at later dates. All were negative.

An antigen prepared from the *Listerella* strain by Paterson's method (1939B) was used to determine the agglutinin titer of the heifer's serum every 3 days from the time of inoculation to 3 days after abortion. During this time agglutination did not occur in dilutions as low as 1 to 50. A *Listerella* rapid-plate antigen prepared by a modification of the method used by Schoening and Creech (1933) for *Erysipelothrix rhusiopathiae* was used in subsequent tests. By this method a titer of 1-3,200 (partial) was obtained 10 days after abortion,

a titer of 1-6,400 (1-7,200 partial) 3 weeks after abortion, and a titer of 1-50,000 a month later.

Blood sugar, total blood ketones (as acetone), serum calcium, and serum inorganic phosphorus were normal on the day of abortion.[1] Total ketone bodies (as acetone) in the urine were slightly high, (55.3 mg. per 100 cc.), probably as a result of the heifer's fast.

Bacteriologic cultures were made of the cerebrum, medulla, heart blood, thymus, and of the marrow of the radius, humerus, femur, and tibia of the aborted fetus. Plain agar plates, meat mash, brain mash, and serum broth were used as the media. *Listerella* was isolated thru most of the media from every site except the marrow of the humerus. Two rabbits and 2 guinea pigs were inoculated subcutaneously with a saline suspension of the cerebrum, but were released healthy one month later.

The placenta was cultured and a saline suspension of selected cotyledons was inoculated subcutaneously into rabbits and guinea pigs. The colostrum was cultured and inoculated subcutaneously and intraperitoneally into rabbits and guinea pigs. Vaginal swabs repeatedly made from the heifer after abortion were cultured bacteriologically. In no case was *Listerella* isolated, and all the inoculated animals remained healthy.

Since this *Listerella* strain appeared capable of causing abortion in cattle, it was considered desirable to determine whether it could do so in other species of animals. Pregnant rabbits, guinea pigs, and swine were utilized in this study.

One pregnant rabbit was inoculated intravenously with .5 cc. of a McFarland Nephelometer Tube 2 saline suspension of this *Listerella* strain. She died 2 days later without aborting and *Listerella* was isolated from her heart blood and medulla. Two other pregnant rabbits were then given subcutaneously 1 cc. of *Listerella* suspension. They gave birth to normal, healthy young 5 and 6 days later.

Three pregnant guinea pigs were inoculated subcutaneously with 1 cc. of a McFarland Nephelometer Tube 2 saline suspension of the *Listerella* strain. On the next day 1 guinea pig gave birth to 2 fully developed dead guinea pigs, but it is probable that the death of the young was not caused by *Listerella.* Their heart blood and brains were negative to *Listerella* on culture. The dam died a week after inoculation, and *Listerella* was recovered from both the heart blood and brain. The other 2 pregnant guinea pigs did not abort, but died, one 8 days and the other 10 days after inoculation.

Two pregnant gilts were inoculated intravenously one with .5 cc. and the other with 1 cc. of a McFarland Nephelometer Tube 4 saline suspension of the same *Listerella* strain. The next day the gilt inocu-

[1]These analyses were made by Dr. Jesse Sampson, Professor of Animal Pathology and Hygiene, University of Illinois.

lated with .5 cc. of the suspension had a temperature of 103.4° F., appeared depressed, and ate little, but temperature and appetite were normal the following day. Nine days after treatment this animal farrowed 11 apparently normal pigs, 2 of which died the next day. *Listerella* was not found on bacteriologic examination of the heart blood of the dead pigs, and uterine and vaginal swabs of the gilt were negative to *Listerella.* On the day of inoculation the gilt's total erythrocyte count was 6,150,000 per cu. mm. The next day it had dropped to 4,330,000, gradually returning to the original level during the next week. The total leucocyte count decreased slightly after treatment, and the differential count fluctuated irregularly. There was no monocytosis.

The second gilt, which had received the 1-cc. dose, had a temperature of 105° F. and failed to eat on the day after treatment. The temperature returned to normal the next day, and the following day her inappetence disappeared. The total leucocyte count, 16,400 at the time of inoculation, decreased progressively to 9,400 one week later. It then returned during the next week to the pretreatment level. No significant variation was noted in the total erythrocyte count or in the differential leucocyte count. The gilt farrowed 9 normal, healthy pigs 15 days after inoculation.

On the day the 2 gilts were inoculated, agglutination tests were carried out with an antigen prepared by Paterson's method. Both gilts exhibited a partial agglutination in a titer of 1-100. Further agglutination tests were made with the rapid-plate antigen. On the day after inoculation the titer of the first gilt was 1-100 and that of the second, 1-50. Sixteen days later the first animal had a titer of 1-1,600 (partial), and the second, 1-200.

In order to compare the effect of this *Listerella* strain from the aborted fetus on a nonpregnant animal with that on the pregnant heifer, an unbred heifer was inoculated with the same amount of *Listerella* culture as had been used on the pregnant heifer. During the next 6 days the unbred heifer was markedly depressed and refused feed. On the seventh day she drank a little water. On the eighth day the animal ate a little rolled oats and recovered gradually thereafter. Eleven days after inoculation the heifer was thin but ate and drank normally and eventually recovered completely. Her temperature was high, between 104.2° F. and 106.6° F., for the first 6 days after inoculation and then returned to normal. The total erythrocyte count remained quite constant during the whole period, but the total leucocyte count decreased slightly. Agglutination studies carried out with Paterson's antigen failed to reveal an increase in agglutinins.

A 6-week-old pig was inoculated intravenously with 1 cc. of a McFarland Nephelometer Tube 10 saline suspension of the *Listerella* strain from the aborted fetus. The next day the temperature of the pig

was 105.4° F., and it died 3 days after treatment. *Listerella* was recovered from the medulla but not from the heart blood.

Third outbreak. Early in May, 1939, a 500-pound Hereford heifer was brought to the Animal Pathology and Hygiene laboratory from a herd of 14 feeder cattle in east-central Illinois. The animals had been shipped into Illinois during the winter, but their origin was unknown. They had been fed shelled corn, a commercial concentrate, soybean hay, and poor quality silage from a pit silo.

Two of the cattle became sick 4 days apart. The local veterinarian reported that the animals staggered, walked thru fences, and fell to the ground, where they lay on their sides. No other animals in the herd were affected.

No gross lesions were observed at autopsy. Cultures from the cerebrum and medulla were made on liver agar plates, and *Listerella* was isolated from the medulla. A rabbit inoculated intravenously with a saline suspension of the organism died 2 days later and *Listerella* was recovered from its heart blood. Two chickens were inoculated intravenously with the same suspension. One died 5 days later and *Listerella* was isolated from its brain and heart blood; the other bird remained healthy. A guinea pig died 9 days after intraperitoneal inoculation with the same organism and the organism was recovered.

Fourth outbreak. Early in July, 1939, an outbreak of encephalitis was reported in a herd of approximately 30 dairy cattle in northern Illinois. Two mature cows were affected. They were refractory when handled and pushed their heads against the manger. The head of one had been found negative to rabies by a public health laboratory in Chicago, and the head of the other animal was brought to the Animal Pathology and Hygiene laboratory for study.

FIG. 5.—CULTURE OF LISTERELLA FROM MEDULLA OF A COW NATURALLY INFECTED

The culture was incubated for 48 hours at 37° C. on plain agar.

No significant gross lesions were observed. Negri bodies were not found upon microscopic examination of the hippocampus. Cultures were made on plain agar plates and in meat mash, brain mash, and a 10-percent serum broth of the cerebrum and medulla. *Listerella* was recovered only from the medulla and on plain agar (Fig. 5).

A saline emulsion of the medulla was inoculated subcutaneously into 2 rabbits and 2 guinea pigs. One rabbit died 2 days later and 1 guinea pig 6 days later. Their heart blood and brains were negative to *Listerella* upon bacteriologic examination.

Fifth outbreak. In January, 1941, a live steer (Fig. 6) was sent to the laboratory from a herd of feeder cattle in western Illinois. It came from the same locality as the fourth outbreak of listerellosis in sheep (page 13), and was under the supervision of the same veterinarian.

The owner first noted the steer's illness 2 days before it was brought to the laboratory. At this time it had difficulty in rising, was unsteady when on its feet, and ate relatively little. The local veterinarian was called the next day and reported that the steer was not only weaving but also circling to the right when it walked. Its temperature was 104.2° F., it refused feed, and the feces were passed as small pellets. A light laxative and antiferments were administered, and 350 cc. of a 50-percent glucose solution was given intravenously. The owner was advised that, if the condition was not a digestive disturbance, it might be listerellosis.

FIG. 6.—STEER AFFECTED WITH LISTERELLOSIS IN FIFTH OUTBREAK
Note attitude of depression and strabismus.

The steer was alive upon arrival at the laboratory. The body temperature was 103.4° F., the total leucocyte count 9,000, and the total erythrocyte count 7,800,000 per cu. mm. It was given 360 grains of sulfanilamide orally and 25 cc. of bovine *Listerella* antiserum intravenously. The following day 300 grains of sulfanilamide were administered by mouth. The animal failed to respond and died on the sixth day of illness, 4 days after arrival at the laboratory.

At the autopsy, evidence of dehydration was observed. The material in the rumen was very dry and quite well packed. Congestion of the blood vessels of the brain and meninges was noted. There were no other gross lesions.

The medulla, cerebrum, hippocampus, and meninges were cultured on plain agar plates and in meat mash, but *Listerella* was isolated only from the medulla and on plain agar. Four guinea pigs inoculated subcutaneously with .2 cc. of a saline suspension of the medulla remained healthy. Each animal of the herd was then vaccinated with 5 cc. of a formalin-killed broth culture of *Listerella.* There were no further losses, but the same result might have been obtained without bacterin.

FIG. 7.—FEEDER CALF AFFECTED WITH LISTERELLOSIS IN SIXTH OUTBREAK
Note the drooling and the drooped ear.

Sixth outbreak. Late in January, 1941, 2 calves were brought to the laboratory from a herd of 40 western feeder animals in north-central Illinois. They had been bought on December 1 and fed corn and a mixed hay. Three animals became sick in the herd at intervals of one day and exhibited symptoms of encephalitis, according to the local veterinarian.

One calf, which had become ill the previous day, was dead on arrival at the laboratory. The other calf (Fig. 7) had first shown symptoms on the day it was brought to the laboratory. The dead calf exhibited opacity of the cornea of the right eye and, at autopsy, showed congestion of the viscera and an excessive amount of fluid in the cranial cavity. The medulla was cultured on plain and blood agar plates and in meat mash. *Listerella* was isolated on the solid media.

The live calf had been given 120 grains of sulfanilamide by the local veterinarian that morning and was given an additional 380 grains at the laboratory on the same day. That evening its temperature was 107.2° F.; the cornea of the right eye was opaque; and the animal circled to the left, seeming unable to turn to the right. The calf received 350 grains of sulfanilamide on each of the next 2 days, but its condition became progressively worse, and it was destroyed when moribund 4 days after arrival. The medulla was cultured on blood agar and plain agar plates, but *Listerella* was not isolated.

A formalin-killed *Listerella* broth culture was prepared and sent to the local veterinarian. He inoculated the remainder of the herd subcutaneously, using two 3-cc. doses each 4 days apart. No further cases were reported.

Seventh outbreak. Late in January, 1942, an outbreak of encephalitis appeared in the cattle on two adjacent farms in northern Illinois. In one herd containing 173 yearling beef cattle heifers (Fig. 8) 11 animals became sick. The animals that died were ill for 3 days. According to the owner, they were dizzy and looked up continually. They had been receiving corn and cob meal, silage, cotton seed, and straw, and no treatment had been attempted at this time.

The other herd contained 110 younger beef cattle, of which 2 died and 1 recovered. No specimens were received at the laboratory from this herd, but since the symptoms were similar, it is presumed that the disease was the same.

At autopsy, one heifer from the first herd showed no gross lesions other than a peculiar mottling of a small area of muscle over one shoulder and an excessive amount of cerebrospinal fluid. The heart blood, liver, and spleen were cultured on blood agar plates, and negative results were obtained. *Listerella* was isolated from the medulla on blood and plain agar plates.

A total of 11 animals became sick in this herd. Of these, 4 died and 1 was destroyed at the laboratory. The local veterinarian admin-

FIG. 8.—HERD IN WHICH THE SEVENTH OUTBREAK OF LISTERELLOSIS OCCURRED

istered massive doses (1,000 grains daily) of sulfanilamide to the rest of the sick animals and they recovered.

Eighth outbreak. Late in May, 1942, a white-faced heifer was brought to the laboratory from a herd of 20 animals in central Illinois. She was the only animal affected and was first seen to be sick on the previous day. At that time she was unable to drink and the veterinarian gave her water thru a stomach tube. Her temperature was 101° F. on that day and also on the next.

After arrival at the laboratory the heifer was given repeated injections with 40-percent glucose. These injections had no apparently beneficial effect and she died the next evening. At autopsy no gross lesions were observed. Results of microscopic examination of the hippocampus were negative to Negri bodies, but *Listerella* was isolated from the medulla on plain agar plates.

Plant poisoning or rabies had been suspected in this case, but the

TABLE 3.—LOSSES FROM LISTERELLOSIS IN OUTBREAKS AMONG CATTLE IN ILLINOIS

Outbreak No.	Animals in herd	Animals dead		Outbreak No.	Animals in herd	Animals dead	
		Number	Percent			Number	Percent
1	68	3	4.4	6	40	3	7.5
2	..	1 (abortion)	...	7 (1st herd)	173	5	2.9
3	15	2	13.3	(2d herd)	110	2	1.8
4	30	2	6.7	8	20	1	5.0
5	..	1	...				

isolation of *Listerella* indicated listerellosis. No other animals in the herd were affected.

Summary of outbreaks among cattle. Losses due to listerellosis in the eight bovine outbreaks described above are summarized in Table 3. From 1.8 to 13.3 percent of the cattle in different herds died of the disease. The average loss in the seven herds for which information was obtainable was 5.9 percent, but if weighted to allow for

FIG. 9.—ILLINOIS COUNTIES IN WHICH OUTBREAKS OF LISTERELLOSIS OCCURRED
The figures indicate the number of outbreaks. **C** stands for an outbreak in cattle, **Ch** in chickens, **G** in goats, and **S** in sheep.

the different numbers of animals in each herd, the average loss becomes 3.95 percent.

The outbreaks of bovine listerellosis recognized are distributed over the state except in the southern part where relatively few cattle are raised (Fig. 9).

Chickens

Listerella infection has been recognized in only one flock of chickens in Illinois (Hurt, Levine, and Graham, 1941).

In November, 1940, a dead white Leghorn pullet about 7½ months old was brought to the laboratory for diagnosis. It belonged to a flock

of 300 birds in eastern Illinois. An outbreak of coryza had occurred when the birds were approximately 3 months old, and about half of the flock had died before the assistance of the laboratory was requested. At this time the death rate was about 15 chickens a week.

At autopsy the viscera of the Leghorn pullet appeared congested. A large amount of thick mucus indicative of coryza was present in the sinuses. The heart blood, liver, and spleen were cultured on blood agar plates, and *Listerella* was isolated from the spleen. Serologic study indicated that the strain belonged to Type 2 of Julianelle and Pons (1939B), the so-called "ruminant" type. A saline suspension of the organism caused conjunctivitis and keratitis when inoculated supraconjunctivally in a rabbit and a guinea pig.

Since it seemed desirable to study this flock more carefully, 6 live sick birds were obtained. Autopsy revealed the presence of a number of diseases. Three of the birds had coryza, and 1 had ocular lymphomatosis. Two birds were severely affected with intestinal coccidiosis, and 2 others had less pronounced infections. *Ascaridia* and tapeworms (primarily *Raillietina cesticillus*) were found in greater or lesser numbers in 5 of the birds. Inflammation of the intestinal mucosa was present in all 6 chickens. The heart blood, liver, and spleen of all birds were cultured on blood agar plates, and the brains of 3 in meat mash. All cultures were negative to pathogenic bacteria.

Three other flocks of chickens raised on other farms but obtained from the same hatchery were free of disease. It is probable that *Listerella* was a secondary invader in the first flock. The presence of the other diseases and parasites tends to support this view, as do the findings of Paterson (1937, 1939A), who reported that pullorum disease and a heavy tapeworm infestation were present in one of the flocks in which he encountered *Listerella* and neurolymphomatosis was present in another.

EPIZOOLOGY OF LISTERELLOSIS

Very little is known as yet regarding the epizoology of listerellosis in domestic animals. The observations of the authors and those of other investigators indicate that in ruminants the disease does not ordinarily sweep thru the herd and affect a high percentage of animals, but rather it runs a more leisurely course, usually affecting less than 10 percent of the herd over a period of several months. Isolated single cases may also appear.

While the morbidity is thus relatively low, the mortality in affected animals is very high. Few animals which have presented symptoms of the disease recover. Young animals appear to be more susceptible than adults, and the disease seems to run a more rapid course in them. Sheep are apparently more susceptible than cattle, since the death

losses among sheep were higher, and death often followed a shorter illness.

Outbreaks of listerellosis have been reported from every part of Illinois but more frequently from the central and northern parts than from the southern third (Fig. 9). Probably this is because there are fewer sheep and cattle in the southern part of the state. Also, this area is at a greater distance from the University.

Listerellosis has been recognized in fifteen Illinois counties. With two exceptions, only a single outbreak was reported in each county. A recurrence of the disease has been reported in two flocks. This wide but sporadic occurrence suggests that the ruminant may not be the usual host of the organism or that the organism may be present in flocks and herds without causing disease, but this is mere speculation. So far no cases of human listerellosis have come to the attention of the Illinois Station.

Gill (1931, 1933, 1937) has suggested that the sheep nasal fly, *Oestrus ovis,* might be concerned in the transmission of ovine listerellosis, but no evidence has yet been presented to prove this hypothesis. The absence of the larvae in some affected sheep, together with the occurrence of the disease in cattle and in spring lambs, suggests that other factors may be involved.

On the farm where the third ovine outbreak occurred, it was noted that there were many rats in the barns during the winter when the disease first appeared. The next winter, when no listerellosis occurred, there were relatively few rats, but rats were again numerous the following year when the disease reappeared. On the farm where the fifth outbreak of ovine listerellosis occurred, no livestock had been kept for several years previous to the time when the disease appeared, but the farm buildings were overrun with rats. Olafson (1940) has suggested that the rat may be a carrier of the disease. In order to check this possibility, in January, 1941, 5 live rats and 2 dead ones were brought to the laboratory from the premises where the third outbreak had occurred. At autopsy no gross lesions were observed on any of the rats. The heart blood, liver, pharynx, and colons of all 7 rats and the lungs of 2 of them were cultured on plain agar plates, but no *Listerella* was recovered. This preliminary study does not, however, eliminate the possible role of the rat in carrying listerellosis, and further investigation would be worth while.

Listerellosis usually occurs in the winter and early spring, a time when the sheep or cattle are confined in barns for all or a part of the time. Losses subside and the disease disappears after the animals are placed on pasture. These facts indicate that crowding has much to do with the transmission of the disease. On the other hand, Olafson (1940) has suggested that feeding dry feeds during the winter lowers resistance sufficiently to allow the organism to get a foothold.

The avenues of infection in ruminants have not been determined, but it is possible that *Listerella* enters the nasal passages. Olafson (1940) considers that listerellosis starts as a rhinitis. This supposition is partially supported by the fact that experimental listerellosis, similar in all its aspects to the natural disease, is not ordinarily produced by intravenous or subcutaneous inoculation or by feeding. Also, Pons and Julianelle (1939) report that an organism which was probably *Listerella* was isolated from the throat of a girl whose blood contained *Listerella.* Gill (1933) reported that repeatedly drenching sheep with the culture by way of the nostril resulted in meningitis and encephalitis. On the other hand, Julianelle (1940, 1941A) has reported that instilling or spraying the culture into the noses of rabbits and monkeys failed to produce the disease; whereas feeding the culture to mice in place of drinking water caused death in all cases. Further investigation is needed to establish the mode of infection, particularly in farm animals.

BACTERIOLOGIC STUDIES

Many investigators have studied the morphologic and biochemic characters of *Listerella;* for example, Seastone (1935), Webb and Barber (1937), Schultz, Terry, Brice, and Gebhardt (1938), Barber (1939), Biester and Schwarte (1939), Julianelle (1940, 1941A), Graham, Hester, and Levine (1940B), Cole (1941), and Harvey and Faber (1941A, 1941B). Probably the most extensive study is that of Harvey and Faber (1941B) who reported the characteristics of fifty strains of *Listerella* from animal and human sources. More recently investigations have been conducted on the specific growth requirements of the organisms by Porter and Pelczar (1941) and Hutner (1942).

The strains isolated at the Illinois Station are small, beta-hemolytic, Gram-positive rods, 1 by .5 microns in size (Fig. 10). In hanging-drop preparations the species exhibits a rather peculiar tumbling motility that appears to be characteristic. These strains grow well both at 37° C. and at room temperature. This ability to produce heavy growth in dextrose broth at room temperature, first noted by Paterson (1939B), is helpful in recognizing them.

Six ovine and four bovine strains of *Listerella* isolated at the Illinois Station and an ovine strain isolated by Jungherr and a bovine strain from Seastone were tested for fermentation reaction (Table 4). All the strains that fermented carbohydrates did so without the formation of gas. Of the pentoses, rhamnose was fermented by all strains; a small amount of acid was produced in xylose by a single strain; and arabinose was not fermented. The monosaccharides dextrose and levulose were fermented by all strains, and a small amount of acid was formed in galactose by one ovine and two bovine strains. All four

disaccharides studied—lactose, maltose, sucrose, and trehalose—were fermented by all *Listerella* strains. The trisaccharide raffinose was not fermented. Of the polysaccharides, acid was produced from dextrin by all strains, while inulin was not attacked. The hexahydric alcohols, dulcitol, mannitol, and sorbitol, and the cyclic alcohol, inositol, were not fermented. All strains produced acid from the glucoside salicin.

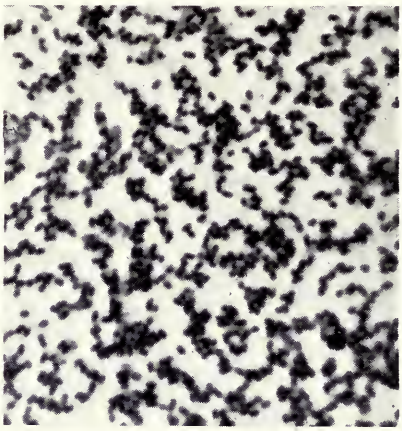

FIG. 10.—CAUSATIVE ORGANISM OF LISTERELLOSIS, *Listerella monocytogenes* (magnified 1200 ×).

The fermentation of some of these compounds, such as lactose, is slower than that of others. Furthermore, sucrose was not fermented by some strains of *Listerella* upon first isolation, but was attacked after a number of transfers on artificial culture media.

The strains studied neither produced indol or acetyl-methyl-carbinol, nor reduced nitrates to nitrites, nor were capable of utilizing the citrate ion as the sole source of carbon.

Many different organs have been cultured in natural cases of *Listerella* encephalitis in ruminants, but the organism has been isolated only from the central nervous system, and indeed in the great majority of cases, only from the medulla. In this respect the natural disease differs from the disease produced experimentally by intravenous inoculation, in which the organism may often be isolated from the blood stream.

Difficulty in obtaining *Listerella* from the brains of naturally affected animals has been reported by some investigators. As pointed out by Biester and Schwarte (1941A), the difficulty was probably due to the small amount of inoculum used. If only a standard loop is used, varying results will be secured. The routine procedure at the Illinois

TABLE 4.—FERMENTATION REACTIONS OF LISTERELLA STRAINS[a]

Strain	Origin	Days incubated	Monosaccharides						Disaccharides				Trisaccharides	Polysaccharides		Alcohols				Glucoside
			Pentoses			Hexoses										Polyhydric			Cyclic	
			Arabinose	Rhamnose	Xylose	Dextrose	Galactose	Levulose	Lactose	Maltose	Sucrose	Trehalose	Raffinose	Dextrin	Inulin	Dulcitol	Mannitol	Sorbitol	Inositol	Salicin
10957-58	Ovine	14	−	+	−	+	−	+	±	±	+	+	−	+	−	−	−	−	−	+
11080-82	Ovine	14	−	+	−	+	−	+	+	+	+	+	−	+	−	−	−	−	−	+
11343	Ovine	14	−	+	−	+	±	+	+	+	+	+	−	+	−	−	−	−	−	+
26045	Ovine	9	−	+	±	+	−	+	+	+	+	+	−	+	−	−	−	−	−	+
26307	Ovine	9	−	+	−	+	−	+	+	+	+	+	−	+	−	−	−	−	−	+
26760	Ovine	9	−	+	−	+	−	+	+	+	+	+	−	+	−	−	−	−	−	+
12159	Bovine	14	−	+	−	+	±	+	+	+	+	+	−	+	−	−	−	−	−	+
12160	Bovine	14	−	+	−	+	±	+	+	+	+	+	−	+	−	−	−	−	−	+
27681	Bovine	9	−	+	−	+	−	+	+	+	+	+	−	+	−	−	−	−	−	+
28164	Bovine	9	−	+	−	+	−	+	+	+	+	+	−	+	−	−	−	−	−	+
Jungherr	Ovine	12	−	+	−	+	−	+	+	+	+	+	−	+	−	−	−	−	−	+
Seastone	Bovine	14	−	+	−	+	−	+	+	+	+	+	−	+	−	−	−	−	−	+

[a]A + sign indicates acid; a − sign, no acid; ± indicates slight acid. No gas was formed in any of the fermentations.

Station is to cut off the head of the specimen at the atlanto-occipital junction and sear the exposed surface of the cephalic end of the spinal cord and the surrounding area. Then sterile forceps and scissors are used to work thru the foramen magnum in order to remove pieces of the medulla. These pieces of medulla are handled with forceps as they are smeared heavily on plates or dropped into liquid media.

Culture media vary in their effectiveness in isolating *Listerella.* The organism grows quite well on plain agar; small colonies, bluish by transmitted light, can readily be distinguished on it in 24 hours. The organism grows still better on blood agar; a clear hemolytic zone, which helps to differentiate the colonies, is present. Incidentally, according to Julianelle (1940), the strains isolated from ruminants grow more luxuriantly than those isolated from man. Liquid media, such as meat mash, may also be employed by plating out on plain agar after 24 to 48 hours' incubation. At the Illinois Station, however, such media have not been so efficient as solid media. In 13 natural cases of listerellosis among sheep and cattle, for which cultures were made upon both solid and liquid media, *Listerella* was isolated from both kinds of media in 6 cases, from solid media alone in 6 cases, and from liquid media alone in only 1 case. The liquid media probably showed *Listerella* less often because it was overgrown with contaminants. This is particularly likely to occur if cultures are made from a head which has been cut off before being shipped to the laboratory.

In isolating *Listerella,* the subcutaneous inoculation of rabbits or guinea pigs with saline suspensions of the medulla has been found to be entirely unsatisfactory. Biester and Schwarte (1941A) have had good results with intracerebral inoculation and, if animal inoculation is desired, this would be the preferred method.

SEROLOGIC STUDIES

The serologic relations of strains of *Listerella* isolated from various sources have been reported upon by a number of investigators, including Seastone (1935), Webb and Barber (1937), Paterson (1939B, 1940A), Julianelle and Pons (1939B), Julianelle (1940, 1941A, 1941B, and 1941C), and Savino and Villazon (1941). In a study of 22 strains, Julianelle (1940) divided the species into two serologic types. Type 1, which he designated as the rodent type, included 2 strains from the rabbit, 1 from the gerbille, 1 from the cow (Strain 27681 isolated by the Illinois Station from an aborted fetus in the second bovine outbreak described previously), and 4 from man. Type 2, which he designated as the ruminant type, included 7 strains from cattle, 2 from sheep, 1 from the goat, 1 from the fox, and 3 from man.

In a study of 54 strains, Paterson (1940A) recognized four types

of *Listerella*. Type 1, characterized by somatic antigens I, II, III (minor), and flagellar antigens AB, included 20 strains from rodents, man, and chickens. It corresponds to Type 1 of Julianelle and Pons. Type 2, characterized by the same somatic antigens as Type 1 and flagellar antigens BD, included a single strain isolated by Gibson (1935) from a case of human meningitis in Scotland. Type 3, characterized by somatic antigens II, IV, III (minor), and flagellar antigens AB, included 10 human strains isolated by Nyfeldt (1929, 1932) and Schmidt and Nyfeldt (1938) from cases of human infectious mononucleosis in Denmark. Type 4, characterized by somatic antigens V, III (minor), and flagellar antigens ABC, included 23 strains isolated from cattle, sheep, chickens, the goat, the fox, and man. This type corresponds to ruminant Type 2 of Julianelle and Pons.

Paterson placed the Illinois Station Strain 27681 from the aborted bovine fetus in Type 4. However, since Julianelle (1940) had confirmed the finding at the Illinois Station and agreed that it belonged in Paterson's Type 1, it was thought that a mistake might have been made in labelling the culture. Accordingly, another transfer was sent to Paterson, who reported in a letter that this must have been the case, since the new transfer clearly fell into his Type 1. To date, this seems to be the only ruminant strain in Paterson's Type 1. All the others isolated at the Illinois Station were Type 2, of Julianelle and Pons, as was the single strain isolated from the chicken.

Agglutination Studies With Uninoculated Cattle

In order to obtain information on the value of the agglutination test in the recognition of animals affected with listerellosis, a study of the agglutinin titers of supposedly healthy cattle was initiated. For this purpose bovine sera sent to the laboratory for the agglutination test for brucellosis were employed. In each case the antigen was prepared from *Listerella* Strain 27681.

Series 1. In this experiment 57 bovine sera negative to *Brucella* agglutinins were used. The antigen was prepared by the method described by Paterson (1939B). The organism was grown in 1-percent dextrose broth for one day at room temperature and then killed with .25-percent formalin. Dilutions of 1-50 to 1-1,600 were set up. The agglutination reactions were read after 24 hours' incubation at 37° C. Agglutinin titers ranged from 1-50 to 1-800, most of the animals giving complete agglutination at 1-200 (Table 5). The 2 sera agglutinating completely at 1-100 gave partial agglutination in a titer of 1-400, as did 7 of the 34 sera agglutinating completely at 1-200. One of the 15 sera agglutinating completely at 1-400 gave partial agglutination at 1-800. Thus partial agglutination reactions were observed with 10 of the 57 sera.

TABLE 5.—TITERS OF COMPLETE AND PARTIAL AGGLUTINATION OF 57 BOVINE SERA TESTED FOR LISTERELLOSIS (SERIES 1)

(Tube antigen grown in 1-percent dextrose broth for 24 hours at room temperature and killed with .25-percent formalin; sera from cattle negative to brucellosis)

Titer of agglutination	Number of sera	Number of sera giving partial agglutination at higher titer	Titer of partial agglutination
1-50	1	0	.....
1-100	2	2	1-400
1-200	34	7	1-400
1-400	15	1	1-800
1-800	5	0	.....
1-1,600	0	..	.....

Series 2. In this experiment 47 bovine sera positive or suspicious to the agglutination test for brucellosis were used. The antigen was prepared as in Series 1. Dilutions of from 1-50 to 1-1,600 were again set up, and the agglutination reactions read as in Series 1. Agglutinin

TABLE 6.—TITERS OF COMPLETE AND PARTIAL AGGLUTINATION OF 47 BOVINE SERA TESTED FOR LISTERELLOSIS (SERIES 2)

(Tube antigen grown in 1-percent dextrose broth at room temperature and killed with .25-percent formalin; sera from cattle positive or suspicious to brucellosis)

Titer of agglutination	Number of sera	Number of sera giving partial agglutination at higher titer	Titer of partial agglutination
1-100	0	0	.....
1-200	3	0	.....
1-400	20	0	.....
1-800	20	0	.....
1-1,600	4	..	.....

titers ranged from 1-200 to 1-1,600, with most of the animals giving complete agglutination in titers of 1-400 and 1-800 (Table 6). No partial reactions were observed.

Series 3. In this experiment 50 bovine sera negative to the agglutination test for brucellosis were used. A rapid-plate antigen was prepared by growing the organism for 24 hours at 37° C. in 1-percent dextrose broth and killing it with .25-percent formalin, centrifuging the organisms, resuspending them in physiological salt solution containing .25 percent of formalin, and adjusting the density of the antigen to 75 times that of the McFarland Nephelometer Tube 1. Dilutions of 1-50 to 1-1,600 were again set up, and the reactions were read after 8 to 10 minutes on the warm plate. Complete agglutinin titers ranged from 1-50 to 1-800, with most animals giving complete agglutination

TABLE 7.—TITERS OF COMPLETE AND PARTIAL AGGLUTINATION OF 50 BOVINE SERA TESTED FOR LISTERELLOSIS (SERIES 3)

(Rapid-plate antigen grown in 1-percent dextrose broth at 37° C., killed with formalin, centrifuged, and standardized to 75 times the density of McFarland Nephelometer Tube 1; sera from cattle negative to brucellosis)

Titer of agglutination	Number of sera	Number of sera giving partial agglutination at higher titer	Titer of partial agglutination
Less than 1-50	2	2	1-400
1-50	3	1	1-200
		2	1-600
1-100	10	1	1-400
		5	1-600
		4	1-800
1-200	16	3	1-600
		6	1-800
		7	1-1,600
1-400	13	1	1-600
		3	1-800
		9	1-1,600
1-600	4	1	1-600
		3	1-800
1-800	2	2	1-1,600
1-1,600	0	..	.....

in titers of 1-100 to 1-400 (Table 7). Only 2 sera failed to agglutinate completely in the dilutions employed. Each of the 50 sera exhibited partial agglutination in titers beyond that at which complete agglutination took place. Indeed, a total of 21 sera gave partial agglutination at 1-1,600, the highest dilution employed.

Series 4. In this experiment 51 bovine sera positive or suspicious to the agglutination test for brucellosis were used. The same rapid-plate antigen was employed as in Series 3, and similar dilutions were set up. Complete agglutination titers ranged from 1-50 to 1-1,600, most animals giving complete agglutination in titers of 1-100 to 1-400 (Table 8). One serum failed to agglutinate completely in the dilutions employed. All sera exhibited partial agglutination in titers beyond that at which complete agglutination took place with the exception, of course, of the serum which agglutinated completely at 1-1,600 and was not tested at a higher dilution. Ten sera gave partial agglutination in a dilution of 1-1,600.

Series 5. In this experiment 35 bovine sera negative to the agglutination test for brucellosis were used. The rapid-plate antigen was prepared and used similarly to that in Series 3, except that it was grown at room temperature and standardized to 50 times the McFarland Nephelometer Tube 1. Dilutions of from 1-50 to 1-800 were set

TABLE 8.—TITERS OF COMPLETE AND PARTIAL AGGLUTINATION OF 51 BOVINE SERA TESTED FOR LISTERELLOSIS (SERIES 4)

(Rapid-plate antigen grown in 1-percent dextrose broth at 37° C., killed with formalin, centrifuged, and standardized to 75 times the density of McFarland Nephelometer Tube 1; sera from cattle positive and suspicious to brucellosis)

Titer of agglutination	Number of sera	Number of sera giving partial agglutination at higher titer	Titer of partial agglutination
Less than 1-50	1	1	1-200
1-50	5	2	1-200
		1	1-400
		2	1-600
1-100	15	2	1-200
		2	1-400
		6	1-600
		4	1-800
		1	1-1,600
1-200	17	2	1-400
		4	1-600
		8	1-800
		3	1-1,600
1-400	10	6	1-800
		4	1-1,600
1-600	2	2	1-1,600
1-800	0	0	.....
1-1,600	1	..	.....

up. Complete agglutination titers ranged from 1-100 to 1-300, most of them being at 1-150 (Table 9). Partial agglutination in titers up to 1-300 were observed with 15 sera.

The sera of two rabbits, one of which had been inoculated intravenously with *Listerella* Strain 27681 (Type 1) and the other with Strain 12159 (Type 4), agglutinated this antigen completely in a di-

TABLE 9.—TITERS OF COMPLETE AND PARTIAL AGGLUTINATION OF 35 BOVINE SERA TESTED FOR LISTERELLOSIS (SERIES 5)

(Rapid-plate antigen grown in 1-percent dextrose broth at room temperature, killed with formalin, centrifuged, and standardized to 50 times the density of McFarland Nephelometer Tube 1; sera from cattle negative to brucellosis)

Titer of agglutination	Number of sera	Number of sera giving partial agglutination at higher titer	Titer of partial agglutination
1-50	0	0	.....
1-100	13	11	1-150
1-150	21	4	1-300
1-300	1	0	.....
1-400	0	0	.....
1-800	0	..	.....

lution of 1-1,600, and partially in a dilution of 1-3,200. The serum of an untreated rabbit produced only partial agglutination in a dilution of 1-50. The sera of two gilts produced partial agglutination in a dilution of 1-50, but 6 days after intravenous inoculation with *Listerella* of Strain 27681 the titer of these gilts had risen to 1-300 (partial).

Series 6. In this experiment 100 bovine sera, which included some negative, positive, and suspicious to the agglutination test for brucellosis, were used. The rapid-plate antigen was prepared and used

TABLE 10.—TITERS OF COMPLETE AND PARTIAL AGGLUTINATION OF 100 BOVINE SERA TESTED FOR LISTERELLOSIS (SERIES 6)

(Rapid-plate antigen grown in 1-percent dextrose broth at 37° C., killed with formalin, centrifuged, and standardized to 75 times the density of McFarland Nephelometer Tube 1; sera from cattle negative, positive and suspicious to brucellosis)

Titer of agglutination	Number of sera	Number of sera giving partial agglutination at higher titer	Titer of partia agglutination
Less than 1-400	94	15	1-400
		5	1-600
		2	1-800
1-400	5	3	1-600
		2	1-800
1-600	1	0	.....
1-800	0	..	.....

as in Series 3, except that dilutions were set up from 1-400 to 1-800. Only 6 of the sera exhibited complete agglutination in these dilutions; 5 of these 6 gave partial agglutination in higher dilutions (Table 10). Of the 94 other sera, 22 agglutinated partially in titers of from 1-400 to 1-800.

Series 7. In this experiment 49 bovine sera negative to the agglutination test for brucellosis were used. The rapid-plate antigen was prepared and used as in Series 3, dilutions of 1-50 to 1-1,600 being employed. Forty-two sera agglutinated completely in dilutions of from 1-50 to 1-1,600 (Table 11). Most of these reactions were in titers of 1-100 to 1-400. The 7 sera which failed to produce complete agglutination in a titer of 1-50 did cause partial agglutination to take place in dilutions ranging from 1-50 to 1-600. Partial agglutination in titers of from 1-100 to 1-1,600 were observed with 37 of the other 42 sera.

Series 8. In this experiment 18 bovine sera were used. They were obtained from animals in the herd in which an abortion had been attributed to *Listerella* Strain 27681. The rapid-plate antigen was prepared and used as in Series 3, dilutions of from 1-50 to 1-1,600 being employed. All animals gave complete agglutination in dilutions from

TABLE 11.—TITERS OF COMPLETE AND PARTIAL AGGLUTINATION OF 49 BOVINE SERA TESTED FOR LISTERELLOSIS (SERIES 7)

(Rapid-plate antigen grown in 1-percent dextrose broth at 37° C., killed with formalin, centrifuged, and standardized at 75 times the density of McFarland Nephelometer Tube 1; sera from cattle negative to brucellosis)

Titer of agglutination	Number of sera	Number of sera giving partial agglutination at higher titer	Titer of partial agglutination
Less than 1-50	7	1	1-50
		3	1-100
		1	1-200
		2	1-600
1-50	5	3	1-100
		1	1-200
		1	1-400
1-100	16	2	1-200
		8	1-400
		2	1-600
		2	1-800
		1	1-1,600
1-200	11	6	1-400
		2	1-600
		2	1-800
1-400	8	2	1-600
		3	1-800
		2	1-1,600
1-600	1	0	.....
1-800	0	0	.....
1-1,600	1	..	.....

TABLE 12.—TITERS OF COMPLETE AND PARTIAL AGGLUTINATION OF 18 BOVINE SERA TESTED FOR LISTERELLOSIS (SERIES 8)

(Rapid-plate antigen grown in 1-percent dextrose broth at 37° C., killed with formalin, centrifuged, and standardized to 75 times the density of McFarland Nephelometer Tube 1; sera from cattle in which abortion occurred from which Strain 27681 was isolated)

Titer of agglutination	Number of sera	Number of sera giving partial agglutination at higher titer	Titer of partial agglutination
1-200	5	1	1-600
		4	1-1,600
1-400	9	4	1-800
		4	1-1,600
1-600	1	1	1-800
1-800	1	1	1-1,600
1-1,600	2	..	.....

TABLE 13.—TITERS OF COMPLETE AND PARTIAL AGGLUTINATION OF 20 BOVINE SERA TESTED FOR LISTERELLOSIS (SERIES 9)

(Tube antigen grown in 1-percent dextrose broth at room temperature, killed with formalin, centrifuged, and standardized to McFarland Nephelometer Tube 1; sera from 20 cattle positive and negative to brucellosis)

Titer of agglutination	Number of sera	Number of sera giving partial agglutination at higher titer	Titer of partial agglutination
1-50	2	0	.....
1-100	5	0	.....
1-200	9	1	1-400
1-400	3	0	.....
1-800	1	0	.....
1-1,280	0	0	.....

1-200 to 1-1,600, most of these agglutinations occurring in dilutions from 1-200 to 1-400 (Table 12). Partial agglutination titers were observed in dilutions ranging from 1-600 to 1-1,600 in 15 of the animals; the sera from 2 of the remaining 3 animals gave complete agglutination in a dilution of 1-1,600, the highest used.

Series 9. In this experiment 20 bovine sera that were either positive or negative to the agglutination test for brucellosis were used. A tube antigen was prepared by growing the *Listerella* culture in .1-percent dextrose broth at room temperature for 24 hours, killing the growth with .5-percent formalin, centrifuging it, and resuspending the organisms in physiological salt solution containing .5 percent of formalin. This suspension was standardized to the density of McFarland Nephelometer Tube 1. Agglutination tests were set up in dilutions of 1-25 to 1-12,800 and were read after 24 hours' incubation at 37° C. Agglutinin titers ranged from 1-50 to 1-800, most of them falling in the 1-100 and 1-200 groups (Table 13). Only one sample gave partial agglutination and this occurred only in the next dilution above that at which agglutination was complete.

Agglutination Studies With Inoculated Heifers

In the pregnant heifer which aborted following a single intravenous inoculation with *Listerella* of Strain 27681, the agglutinin titer was tested from the time of inoculation to a month after abortion. Her titer was less than 1-50 from the time of inoculation until after she aborted. Ten days after the abortion her titer was 1-3,200 (partial) by the rapid-plate method, becoming 1-6,400 three weeks after abortion and 1-50,000 one month after abortion.

Another pregnant heifer was utilized in a study to determine the duration of the agglutination titer and also to learn whether abortion might follow repeated subcutaneous injections with *Listerella*. The

heifer was inoculated subcutaneously at 4- to 5-day intervals with increasing doses of living culture Strain 27681 incubated 48 hours and washed off large serum agar slants. A total of six doses was given. The animal became increasingly inappetent and lost weight gradually. Finally, on the twenty-eighth day after the initiation of the experiment, she aborted. The heifer then regained her appetite and began to gain back the weight she had lost. At the time of abortion her agglutinin titer was 1-4,600 by the rapid-plate test (Table 14); it became 1-6,400

TABLE 14.—TUBE AND RAPID-PLATE TESTS OF LISTERELLA AGGLUTININS FROM A HEIFER REPEATEDLY INOCULATED AFTER ABORTION

(Living *Listerella* Strain 27681 was given subcutaneously; culture was washed off a 48-hour growth from large serum agar slants)

Days after beginning of treatment	Dosage of slants	Tube titer	Rapid-plate titer
0	1	.....	.....
4	2	.....	.....
9	3	.....	.....
13	4	.....	.....
17	5	.....	.....
21	6	.....	.....
28 (abortion)	..	.....	1-4,600
34	..	1-6,400	1-6,400
40	6	1-9,600	1-9,600
45	..	1-12,800	1-12,800
59	..	1-50,000	1-40,000
78	..	1-8,000	1-16,000
102	..	1-4,000	1-8,000
131	..	1-500	1-1,000

on the sixth day after abortion and 1-9,600 on the twelfth day after abortion, according to both the rapid-plate and tube tests. After the last test the heifer was given a subcutaneous injection with the growth from six serum agar slants. Nineteen days later her agglutinin titer had risen to a peak of 1-50,000 by the tube test (1-40,000 by the rapid-plate test). Thereafter the agglutinin titer declined, becoming 1-500 by the tube test (1-1,000 by the rapid-plate test) 91 days after the last inoculation.

This pregnant heifer inoculated repeatedly with subcutaneous injections of *Listerella* gave a response roughly parallel to that of the pregnant heifer inoculated intravenously. The agglutinin titer did not reach the high point until some time after abortion and the high titer lasted only a relatively short time.

Comparison of Rapid-Plate and Tube Agglutination Tests

The rapid-plate and tube antigens were compared by testing the reactions of sera of 15 cows from the same herd. The rapid-plate antigen was prepared and used as in Series 3 (page 36), dilutions

from 1-50 to 1-1,600 being employed. The tube antigen was prepared according to Paterson's method (1939B) but only dilutions from 1-50 to 1-200 were used.

The agglutinin titer was consistently higher with the rapid-plate antigen but there was a low degree of correlation between the titers obtained from the two antigens (Table 15). An animal with a tube-test titer of less than 1-50 had a rapid-plate titer of 1-400. Of the 6 animals with tube-test titers of 1-50, 3 had a rapid-plate titer of 1-200; 1 had 1-400; 1 had 1-600; and the other, 1-800. Of the 4 animals with tube-test titers of 1-100, 2 had rapid-plate titers of 1-400, and the other 2 had rapid-plate titers of 1-1,600. With the rapid-plate test, partial or incomplete agglutination was observed in all but one case in dilutions above those recorded as complete and ranging from 1-600 to 1-1,600.

In testing both the uninoculated cattle and the inoculated heifers in the experiments described on pages 35 to 42, both tube and rapid-plate antigens were employed. In general, the results obtained with the two antigens in these experiments were comparable to those secured in the experiment just described.

TABLE 15.—TUBE AND RAPID-PLATE TESTS OF LISTERELLA AGGLUTININS OF 15 BOVINE SERA

(Tube antigen prepared by Paterson's method; rapid-plate antigen prepared as in Series 3, page 36)

Cow No.	Tube titer of agglutination	Rapid-plate titer	
		Complete agglutination	Partial agglutination
1	Less than 1-50	1-400	.....
2	1-50	1-200	1-1,600
3	1-50	1-200	1-1,600
4	1-50	1-400	1-800
5	1-50	1-600	1-800
6	1-50	1-200	1-600
7	1-50	1-800	1-1,600
8	1-100	1-400	1-800
9	1-100	1-400	1-800
10	1-100	1-400	1-1,600
11	1-100	1-400	1-1,600
12	1-200	1-400	1-800
13	1-200	1-1,600	.....
14	1-200	1-1,600	.....
15	1-200	1-400	1-1,600

Altho the rapid-plate antigen permits agglutination reactions to be read quickly, it has certain disadvantages. As a rule, the titers obtained with it were higher than those given by the tube antigen. This difference might, of course, be minimized by adjusting the density of the

antigen or the amount of serum used. The difference between the two readings was so large, however, that some discrepancy would still exist even if this adjustment were made. Another and more serious fault of the rapid-plate antigens used in this investigation was that they gave a large number of partial or incomplete reactions. These were usually observed in several dilutions beyond that at which complete agglutination occurred, and they made it difficult to read the tests as accurately as would be desired. Relatively few partial reactions occurred with the tube test. When these factors are taken into consideration, it is felt that the tube test is more satisfactory than the rapid-plate method for studying *Listerella* agglutination reactions.

Agglutination Test Not Diagnostic of Listerellosis

The sera of a relatively high percentage of cattle with no history of exposure to *Listerella* contained agglutinins for the organism. This might indicate either that *Listerella* does not give a specific agglutination or that the infection is widespread in animals which never show symptoms. Further investigation may show which occurs. At present the agglutination reaction cannot be used to diagnose listerellosis. This view is shared by Schwarte and Biester (1942).

No Relation Between Listerella Agglutinin Titer and Periodic Ophthalmia in Horses

That *Listerella* might be associated with periodic ophthalmia in horses was suggested by the fact that conjunctivitis and keratitis were observed in sheep and cattle suffering from listerellosis and that *Listerella* was able to produce lesions similar to these in rabbits and guinea pigs. The eyes of two horses destroyed because of periodic ophthalmia were cultured carefully, but *Listerella* was not isolated.

Blood was obtained from 17 horses with a history of periodic ophthalmia and from 13 horses with no such history. Agglutination tests were carried out with tube antigens prepared from three *Listerella* strains of Paterson's Type 4 according to his method (1939B). Since there had been some discussion in the literature about this time (1939) on the possible relation of *Brucella* to periodic ophthalmia,[1] agglutination tests were also carried out with standard tube antigen of *Brucella abortus.* Dilutions of 1-25 to 1-200 were used with *Listerella,* and from 1-50 to 1-200 with *Brucella.*

No significant difference could be seen between the *Listerella* titers in the horses affected with periodic ophthalmia and those that did

[1]For a review of theories on the etiology of periodic ophthalmia see Jones (1942).

TABLE 16.—LISTERELLA AND BRUCELLA AGGLUTININS IN SERA FROM HORSES WITH A POSITIVE OR NEGATIVE HISTORY OF PERIODIC OPHTHALMIA

Horse No.	History of periodic ophthalmia	*Listerella* agglutinins												*Brucella* agglutinins		
		Strain 10957				Strain 26307				Strain 12159				1-50	1-100	1-200
		1-25	1-50	1-100	1-200	1-25	1-50	1-100	1-200	1-25	1-50	1-100	1-200			
1	+	+	±	−	−	+	+	−	−	+	+	±	−	−	−	−
2	+	±	±	−	−	∓	−	−	−	+	+	−	−	−	−	−
3	+	+	+	∓	∓	∓	−	−	−	+	+	+	±	−	−	−
4	+	+	+	+	−	+	+	−	−	+	+	+	±	+	∓	−
5	+	+	+	+	+	+	+	−	−	+	+	−	∓	∓	−	−
6	+	+	+	+	±	+	+	+	−	+	+	+	+	+	−	−
7	+	+	−	−	−	±	−	−	−	+	−	−	−	−	−	−
8	+	+	−	−	−	+	−	−	−	−	±	−	−	+	+	∓
9	+	−	−	−	−	+	−	−	−	∓	−	∓	−	−	−	−
10	+	+	+	−	−	+	−	−	−	+	+	−	−	−	−	−
11	+	∓	∓	−	−	+	−	−	−	−	−	−	−	−	−	−
12	+	+	+	−	∓	+	+	−	−	∓	+	+	+	−	−	−
13	+	+	−	−	−	+	−	−	−	+	−	−	−	−	−	−
14	+	+	+	−	−	+	−	−	−	±	−	−	−	−	−	−
15	+	+	−	−	−	+	−	−	−	∓	∓	−	−	+	+	−
16	+	+	−	−	−	−	−	−	−	+	∓	−	−	−	−	−
17	+	±	−	−	−	+	+	−	−	+	+	−	−	−	−	−
18	−	∓	+	+	−	+	+	−	−	+	+	+	−	+	−	−
19	−	+	−	−	−	+	+	−	−	+	−	−	−	−	−	−
20	−	∓	+	+	−	±	+	−	−	+	∓	−	−	−	−	−
21	−	−	−	−	−	∓	−	−	−	+	−	−	−	−	−	−
22	−	+	+	−	−	+	−	−	−	+	+	−	−	−	−	−
23	−	−	∓	−	−	+	+	+	+	+	+	+	−	−	−	−
24	−	+	+	∓	∓	+	+	+	−	+	+	−	−	−	−	−
25	−	+	+	+	+	+	+	+	−	+	+	−	−	∓	−	−
26	−	+	+	−	−	+	∓	−	−	+	±	∓	∓	−	−	−
27	−	−	−	−	−	+	∓	−	−	−	−	−	−	−	−	−
28	−	∓	−	−	−	+	+	−	−	+	+	+	+	−	−	−
29	−	∓	+	−	−	+	−	−	−	+	+	∓	∓	−	−	−
30	−	+	−	∓	−	+	+	−	−	+	∓	+	−	−	−	−
Positive serum controls		+	+	+	+	+	+	+	+	+	+	∓	−	+	+	+

not have the disease. The sera from 5 of the affected animals agglutinated the *Brucella* antigen to some degree, as did that of 1 of the horses that did not have periodic ophthalmia (Table 16). No *Brucella* agglutinins were found in the remainder of the horses.

The results obtained with agglutinin titers present no evidence that *Listerella* has any etiologic relationship to equine periodic ophthalmia.

Complement Fixation Tests

An attempt was made to utilize the complement fixation test in the hope that it might be of more value than the agglutination reaction in diagnosing listerellosis. An antigen prepared from *Listerella* Strain 27681 in the same way as that in Series 3 was found to be anticomplementary, altho it was not hemolytic. A similar antigen, in which the bacteria were killed by heating at 60° C. for one hour, was standardized to 40 times the density of the McFarland Nephelometer Tube 1 and preserved with .5-percent phenol. This antigen was found to be neither hemolytic nor anticomplementary but antigen prepared in this manner became anticomplementary in about 3 weeks.

Complement fixation tests were set up with serum from the pregnant heifer inoculated intravenously with *Listerella* of Strain 27681 (page 19) and with serum from a cow with no history of exposure to *Listerella.* The test was positive for the serum from the pregnant heifer and negative for the cow. The sera of 10 rabbits inoculated with *Listerella* were positive; whereas the sera of 5 horses not exposed to the organism were negative. However, when another lot of antigen was used, these same 5 horses were positive on repeated tests, altho the negative control serum was still negative. Similarly, the sera of 3 sheep with no history of listerellosis in the flock from which they originated were positive on repeated tests. Five out of 10 bovine sera which had been sent to the Animal Pathology and Hygiene laboratory for the agglutination test for brucellosis were positive to the complement fixation test for listerellosis; the other 5 were negative. Out of 12 other bovine sera sent in to be tested for brucellosis, 11 were also positive for listerellosis, according to the complement fixation test.

Eleven rabbits were inoculated subcutaneously with *Listerella* of Strain 27681. Their blood was later obtained and a complement fixation test was carried out. Two were positive, 3 gave partial reactions, and the other 6 were negative. Eleven untreated rabbits were negative. The control bovine serum from a heifer repeatedly inoculated subcutaneously with *Listerella* was positive.

From the results just described it was concluded that the complement fixation test would have little value in the diagnosis of listerellosis. Much too large a percentage of supposedly normal sera gave a positive reaction to this test, and serum from inoculated animals sometimes failed to react.

EXPERIMENTAL LISTERELLOSIS

There are a number of reports in the literature on experimental listerellosis both in species of animals in which the disease has been recognized in the field and in species not yet known to be naturally affected. Studies on the artificial production of listerellosis were carried out at the Illinois Station in sheep, cattle, horses, swine, cats, dogs, and chickens, in addition to the rabbit and guinea pig. Most of the work described here has been reported elsewhere by Graham, Dunlap, and Levine (1940).

Sheep

Gill (1933, 1937) reported that 2 sheep inoculated subcutaneously with *Listerella* cultures exhibited no symptoms, but repeated inoculation of culture into their stomachs was followed by a rise in temperature which lasted 3 days. Further intragastric exposure had no effect on these sheep. Intranasal exposure was more successful in producing the disease. Two out of three sheep exposed by this route exhibited cerebral symptoms within a week, and the third sheep had a nasal catarrh and transient fever. Intravenous inoculation of 4 sheep produced a rise in temperature followed by recovery; but intracarotid inoculation was followed by symptoms similar to those in the natural disease and death resulted.

Intranasal inoculation was found by Jungherr (1937) to be followed by a temporary illness in a single sheep, and he reported similar results following conjunctival inoculation. He found that death accompanied by hemorrhagic meningitis took place after intracarotid inoculation. On the other hand, Biester and Schwarte (1939) obtained only a rise in temperature followed by recovery after intracarotid inoculation of a sheep. Intracerebral inoculation caused death, and one sheep died following a series of fifteen subcutaneous and intramuscular inoculations. Working with a bovine strain of *Listerella*, Schwarte and Biester (1942) produced death in 3 sheep by intracranial exposure, and illness accompanied by a febrile reaction appeared in 2 sheep inoculated intravenously. Olafson (1940) reported that intracerebral inoculation of sheep caused acute exudative encephalitis and meningitis followed by death. Intracarotid inoculation was also rapidly fatal, but intravenous or subcutaneous exposure of 20 sheep was followed only by a rise in temperature and temporary illness. Encephalitis could not be produced in any one of 20 sheep by intranasal instillation even after injuring the nares with a small brush. Paterson (1940D) inoculated pregnant sheep intravenously and produced abortion but not death; whereas repeated oral administration produced no effect. Muth and Morrill (1942) caused death in 2 days by intracerebral inoculation of two lambs. Pallaske (1943) failed to infect sheep with culture by way of the naso-cerebral tract, middle ear, and alimentary tract.

Strain 10957 of *Listerella* (Paterson Type 4), isolated from a case of ovine listerellosis in Illinois, was employed for the studies of experimental listerellosis made at the Illinois Station.

Intracerebral inoculation. One lamb was inoculated intracerebrally with .25 cc. of a saline suspension of *Listerella* (density McFarland Nephelometer Tube 1). At the time of inoculation its temperature was 103° F. On the following day the temperature rose to 107.6° F. and the animal trembled, its spine became rigid, and it walked blindly into obstacles. The total erythrocyte count remained unchanged, but the total leucocyte count rose from 8,200 before inoculation to 19,500 on the following day. The leucocytosis was due primarily to a marked increase in neutrophiles and monocytes. The neutrophiles increased from 51 percent before treatment to 88 percent the next day. The lymphocytes decreased correspondingly, from 48 to 6 percent, and the monocytes increased from 1 to 6 percent. The lamb died during the night about 36 hours after inoculation.

At autopsy congestion of the kidneys, lungs, spleen, and mesenteric lymph nodes was noted, but the outstanding lesions were diffuse purulent meningitis and a purulent encephalitis, together with softening and hemorrhage involving particularly the cerebrum.

Bacteriologic cultures were made of the medulla, cerebrum, hippocampus, lumbar cord, heart blood, pericardial fluid, mesenteric lymph nodes, liver, kidney, and spleen. *Listerella* was isolated from the medulla, cerebrum, hippocampus, lumbar cord, lymph nodes, liver, kidney, and spleen.

Intravenous inoculation. Another lamb was inoculated intravenously with 1 cc. of a saline suspension of *Listerella* (density McFarland Nephelometer Tube 10). Weakness, dullness, and frequent urination were observed the following day. On the day after, the rate of respiration had increased and breathing was labored. The lamb became progressively worse and 9 days after exposure was unable to stand. A marked nasal discharge, which persisted until death, appeared 13 days after inoculation. The lamb became comatose and remained in this condition until it died 18 days after inoculation.

On the day of exposure the lamb's temperature was 103° F. It rose to 107.4° F. on the next day and then gradually declined. Ten days after inoculation it was 103.2° F. and remained at about that level until death. No significant change was observed in the total erythrocyte count thruout the illness, but a temporary leucocytosis appeared. The total leucocyte count was 5,450 at the time of inoculation. Six days later it rose to 11,150 and then 10 days after exposure dropped to 8,650. An increase in neutrophiles from 54 percent to 72 percent was observed 12 days after inoculation; the lymphocytes decreased correspondingly from 43 percent to 25 percent; the monocytes remained constant at 3 percent.

Aside from congestion of the medullar blood vessels, no gross lesions were observed in the central nervous system at autopsy.

Bacteriologic cultures were made of the medulla, cerebrum, hippocampus, lumbar cord, heart blood, pericardial fluid, liver, peritoneal fluid, mesenteric lymph nodes, kidney and spleen, but *Listerella* was recovered only from the lumbar cord.

Intravenous exposure also caused the death of 7 out of 8 lambs and all 5 ewes in a vaccination experiment described on page 83.

Supraconjunctival inoculation. One lamb was exposed to *Listerella* by dropping into the eye 1 cc. of a saline suspension of the organism (density McFarland Nephelometer Tube 10). No reaction was observed until the eleventh day, when the lamb appeared weak and walked unsteadily. Thirteen days after exposure a watery discharge came from the eye, and the next day opacity of the cornea was noted. The keratitis and lacrimation began to subside 3 days later, and the eye was apparently normal a month after inoculation. No temperature reaction was observed during this whole period.

In order to determine whether the infection had produced a local immunity, the same eye was exposed as described above one month after the first inoculation, and the inoculation was repeated 1, 2, and 5 days later. Neither lesions nor temperature reaction resulted.

The total erythrocyte and leucocyte counts did not vary significantly after inoculation, but the differential leucocyte count fluctuated somewhat. The neutrophiles tended to decrease and the lymphocytes to increase slightly. Six days after inoculation a very slight monocytosis (9 percent) was present; by 15 days after exposure there was a slight eosinophilia (8 percent).

Ten days after the last inoculation the lamb was destroyed. No gross pathologic changes attributable to *Listerella* were observed, and bacteriologic cultures of the heart blood, liver, and spleen were negative.

Intragastric inoculation. A dose of 10 cc. of a saline suspension of *Listerella* (density McFarland Nephelometer Tube 10) was administered to a single lamb by stomach tube. Two days later the lamb presented an unthrifty appearance and was observed grinding its teeth. The rate of respiration and the temperature had increased. These symptoms lasted for a day or two, after which the lamb returned to normal.

One month after treatment the lamb was given three daily doses of 15 cc. of the *Listerella* culture by stomach tube, and 3 days later a single 45-cc. dose was similarly administered. No symptoms resulted and the temperature remained normal, suggesting that the first exposure may have produced some degree of immunity.

The total erythrocyte count did not change significantly during the

experiment. A transient leucocytosis was noted 2 days after the first feeding, when the total leucocyte count had risen from 7,250 to 12,400. Two days later it was again at the pretreatment level, and it remained there during the remainder of the observation period. The neutrophiles decreased progressively from 60 percent at the time of the first treatment to 30 percent 10 days after the last inoculation. The lymphocytes increased correspondingly from 38 percent to 66 percent. No significant change in monocytes occurred.

The lamb was destroyed 10 days after the last feeding. No gross lesions attributable to *Listerella* were found at autopsy. Bacteriologic cultures were made of the medulla, heart blood, liver, and spleen, but *Listerella* was not isolated.

Contact exposure. Two lambs were exposed to listerellosis by confinement in the same pen with the experimentally infected animals. The exposed lambs showed no symptoms during an observation period of a month.

Cattle

Jones and Little (1934) reported that intracerebral inoculation of *Listerella* in calves caused meningitis and death. Paterson (1940D) produced abortion in a pregnant cow by intravenous inoculation with Illinois *Listerella* Strain 27681. Schwarte and Biester (1942) produced illness but not death in 2 calves by intracranial inoculation with 1 cc. of saline suspensions of *Listerella* (density McFarland Nephelometer Tube 2). Intravenous inoculation with 20 cc. of *Listerella* cultures of similar density produced illness in 2 calves but death did not result. Following recovery the two calves were each given fifteen intravenous doses of 10 to 20 cc. of culture also of similar density, and no further symptoms developed. Two dairy cows, one of which was pregnant, were each inoculated intravenously with 10 cc. of *Listerella* culture. Both became ill, had temperature reactions, and recovered. The pregnant animal gave birth to a normal calf 10 days after inoculation. Oral administration of 250 cc. of *Listerella* culture produced illness and a temperature reaction in one of two calves. After the one recovered, both calves were given fifteen further doses of 200 cc. each and no visible symptoms developed. *Listerella* culture given in sixteen subcutaneous doses of 10 to 20 cc. each failed to produce a temperature reaction or other symptoms in another calf.

In producing experimental listerellosis in cattle at the Illinois Station, *Listerella* Strain 10957 was used. This is the same strain that was used for inoculating the sheep.

Intracerebral inoculation. A Jersey heifer was inoculated intracerebrally with .5 cc. of a saline suspension of *Listerella* (density McFarland Nephelometer Tube 1). The next day she was dull and refused both feed and water; she died the following morning. At the

time of inoculation the temperature of the heifer was 101.6° F., but it rose to 104° F. the next day. She died before her temperature was taken the following day.

At autopsy an acute purulent meningitis was observed. The medulla, cerebrum, hippocampus, lumbar cord, heart blood, pericardial fluid, liver, peritoneal fluid, mesenteric lymph nodes, kidney, spleen, and horn core were cultured, and *Listerella* was recovered from the medulla, cerebrum, hippocampus, lumbar cord, kidney, liver, and spleen.

Intravenous inoculation. A yearling Hereford steer was inoculated intravenously with 2 cc. of a saline suspension of *Listerella* (density McFarland Nephelometer Tube 10). Altho the animal appeared normal the next day, its temperature had risen from 101.4° to 106.8° F. On the following day its temperature was 106° F., the eyes appeared abnormally bright, and the animal refused to drink. During the next 3 days the symptoms disappeared.

A further series of intravenous inoculations was administered one month after the first injection. Three daily doses of 2 cc. each were given, followed after a 3-day interval by a single 6-cc. dose. The temperature rose after the first of these injections but quickly returned to normal and no other symptoms were noticed.

No significant changes were observed in the total erythrocyte and leucocyte counts or in the differential leucocyte count thruout the experiment.

Intravenous exposure of a pregnant heifer to Strain 27681 *Listerella* was followed by illness, abortion, and recovery (pages 19 and 20).

Supraconjunctival inoculation. A yearling Hereford steer was exposed by dropping into the eye .1 cc. of a saline suspension of *Listerella* (density McFarland Nephelometer Tube 10). Its temperature did not vary significantly, but a watery discharge from the eye, together with a mild conjunctivitis, was observed 12 days after exposure. The conjunctivitis became worse, persisted for several days and then subsided.

Three daily instillations into the eye with .1 cc. each of a similar *Listerella* suspension were administered one month after the first exposure. Three days later a fourth instillation was given. Neither ocular symptoms nor temperature reaction followed this later treatment.

No significant change was observed in the total erythrocyte or leucocyte counts during the experiment, but a slight monocytosis (12 percent) was noted 8 days after the first exposure. This had disappeared 2 days later.

A swab culture was made from the eye during the period of conjunctivitis 14 days after the first inoculation, and *Listerella* was recovered.

Intragastric inoculation. A yearling Hereford steer was given

by means of a stomach tube 15 cc. of a saline suspension of *Listerella* (density McFarland Nephelometer Tube 10). Neither visible symptoms nor thermal reaction followed. Three daily doses of 25 cc. each were given in the same way one month after the first treatment, and a fourth dose of 75 cc. was administered 3 days later. Neither visible symptoms nor thermal reaction was observed after this treatment.

Neither the total erythrocyte nor leucocyte counts nor the differential leucocyte counts varied significantly during the course of the experiment.

Horses

There is apparently no record in the literature, other than that of the Illinois Station, on the exposure of horses to *Listerella.* In the Illinois studies the same ovine Strain 10957 was used in exposing horses as had been used in exposing cattle and sheep.

Intracerebral inoculation. One horse was inoculated intracerebrally with .5 cc. of a saline suspension of *Listerella* (density McFarland Nephelometer Tube 1). Its temperature was 101° F. on the day of inoculation and rose to 103.6° F. the next day. The animal was destroyed when moribund on the second day after exposure.

No significant change occurred in the total erythrocyte count but a leucocytosis was noted. The total leucocyte count rose from 10,550 at the time of inoculation to 16,500 on the day the horse was killed. This was due primarily to an increase in neutrophiles from 65 percent to 85 percent. The lymphocytes decreased correspondingly from 32 percent to 12 percent. The monocytes remained unchanged at 3 percent.

At autopsy an acute purulent meningitis was observed. A large area of softening and hemorrhage was present in the cerebrum surrounding the point of inoculation. The cerebellum and medulla were congested. The medulla, cerebrum, hippocampus, lumbar cord, heart blood, pericardial fluid, liver, peritoneal fluid, mesenteric lymph nodes, kidney, and spleen were cultured, and *Listerella* was isolated from the medulla, cerebrum, and hippocampus.

Intravenous inoculation. One horse was inoculated intravenously with 2 cc. of a saline suspension of *Listerella* (density McFarland Nephelometer Tube 10). Its temperature rose to 103.6° F. on the day after inoculation but returned to normal in 2 days, and no other symptoms were observed.

One month later the horse was given a series of three daily intravenous injections of 2 cc. each of a similar *Listerella* saline suspension. Three days later a 6-cc. dose was similarly administered, and 9 days thereafter a single dose of 80 cc. was given. After the first inoculation of this series, a temperature reaction was observed which lasted

for 2 days. After the massive last injection, collapse seemed imminent; the horse perspired heavily and exhibited dyspnea and incoordination. It appeared normal, however, an hour later. Its temperature rose to 103° F. after this last inoculation, where it remained until the animal was destroyed one week later.

The total erythrocyte count did not vary significantly during the course of the experiment but leucocytosis was noted. The total leucocyte count rose from 8,500 before the first inoculation to 13,950 eight days later and then decreased. It was 9,350 on the day of the last injection but rose to 12,400 a week later. No significant change in the differential blood picture was observed, except that 8 days after the initial injection the monocyte count had risen slightly to 9 percent. However, it had returned to the original level 2 days later.

At autopsy the abdominal muscles were edematous. Bacteriologic cultures were made of the cerebrum, hippocampus, medulla, heart blood, liver, and spleen, and *Listerella* was isolated from the heart blood.

A second horse which had been exposed to *Listerella* before by the conjunctival route was given intravenously 60 cc. of a saline suspension of *Listerella* (density McFarland Nephelometer Tube 10); a third animal which had been previously exposed intragastrically (page 54) was given intravenously 80 cc. of a similar suspension of *Listerella.*

The horse that received 60 cc. appeared normal an hour after inoculation. A day later its temperature had risen to 103° F., and it remained high until the eighth day, when the animal was destroyed. No other symptoms were observed. At autopsy edema was observed in the subcutaneous tissues of the abdominal region. A small amount of clear fluid was present in the subdural space. The liver was congested and contained a few discrete, brownish, subcapsular nodules. There were no other gross lesions.

The total erythrocyte count decreased from 4,910,000 on the day of inoculation to 3,760,000 five days later; the total leucocyte count rose from 7,700 to 12,000. A concomitant increase in neutrophiles from 70 percent to 89 percent was observed. No significant change took place in the percentage of monocytes. Bacteriologic cultures were made of the cerebrum, hippocampus, medulla, heart blood, liver, spleen, and mesenteric lymph nodes, but *Listerella* was not isolated.

The horse which had received 80 cc. of *Listerella* suspension trembled severely and collapsed 2 minutes after inoculation. Dyspnea and profuse hemorrhage from the nostril followed, and the animal died in 5 minutes. An autopsy was not performed.

Supraconjunctival inoculation. One horse was inoculated by dropping into the eye .1 cc. of a saline suspension of *Listerella* (density McFarland Nephelometer Tube 10). The next day catarrhal conjunctivitis was observed but the following day the eye appeared normal.

No temperature reaction occurred, and no significant changes were observed in the total erythrocyte and leucocyte counts or in the differential leucocyte counts.

One month later three daily doses of .1 cc. each of the *Listerella* suspension were instilled into the same eye, and a fourth exposure was given 3 days later. Neither symptoms nor temperature reactions were noted, and the total erythrocyte and leucocyte counts and the differential leucocyte counts did not change significantly.

Intragastric inoculation. One horse was inoculated by stomach tube with 25 cc. of a saline suspension of *Listerella* (density McFarland Nephelometer Tube 10). The next day diarrhea was observed, and the horse's temperature rose to 105.4° F. during the following 2 days. It then subsided, and no other symptoms were noted. The total erythrocyte count did not change significantly, but the total leucocyte count decreased from 8,100 before inoculation to 3,300 two days later. It remained low for four more days and then returned to the previous level. Concomitantly the neutrophiles decreased from 65 percent at the time of treatment to 43 percent 4 days later, subsequently returning to the original level. The lymphocytes increased from 30 percent at the time of inoculation to 51 percent 4 days later and then decreased to the previous level. The increase was purely relative, however, since consideration of both the total and differential counts showed an actual decrease in lymphocytes. No significant change in percentage of monocytes was observed.

A series of three daily doses of 25 cc. each of the *Listerella* suspension was administered by stomach tube one month after the first feeding. Three days later the horse was started on three more daily doses of 25 cc. each administered in the same manner. Neither temperature reaction nor other symptoms were noted. An eight-year-old horse was fed with a stomach tube 350 cc. of a broth culture of *Listerella* (density McFarland Nephelometer Tube 5). Three days later a 400-cc. dose was similarly administered. Neither a temperature reaction nor other symptoms were observed, and the total erythrocyte and leucocyte counts and the differential leucocyte counts did not change significantly.

Swine

Biester and Schwarte (1939) caused death in 2 pigs by intracerebral inoculation with an ovine *Listerella* strain. They gave 2 other pigs, which weighed 55 pounds, one intraperitoneal and seventeen intramuscular doses of the same strain; these pigs became ill and were killed when moribund. Working with a porcine strain of *Listerella,* Biester and Schwarte (1940) found that intracerebral inoculation caused death in about 24 hours. Two 120-pound pigs given repeated intramuscular inoculations of the same strain remained healthy, as

did other pigs fed *Listerella* by stomach tube or inoculated intravenously. Schwarte and Biester (1942) caused death in one to two days in 3 pigs inoculated intracerebrally with a bovine strain of *Listerella* and produced temporary illness accompanied by a temperature reaction in 2 other pigs by means of intravenous inoculation.

Listerella Strain 26045 (Paterson Type 4), isolated from an outbreak in a flock of sheep, was used in the Illinois studies of experimental listerellosis in swine.

Intravenous inoculation. A month-old pig was exposed by intravenous inoculation of .5 cc. of a saline suspension of *Listerella* (density McFarland Nephelometer Tube 5). A temperature reaction and

FIG. 11.—PIG WITH LISTERELLOSIS

This pig was intravenously exposed to *Listerella* 7 days before this picture was taken. Note posterior paralysis.

marked depression were observed for 2 days after inoculation. The pig then made a complete recovery.

Another month-old pig was inoculated intravenously with 1 cc. of the same *Listerella* suspension. The next day its temperature was elevated. It remained between 104° and 105° F. for 2 days, and then returned to normal, falling to subnormal level for 3 days before death, which occurred 9 days after inoculation. Posterior paralysis (Fig. 11) was present for several days before death. The heart blood and medulla were cultured and *Listerella* was isolated from both.

Supraconjunctival inoculation. Two month-old pigs were exposed by instilling into the eye .2 cc. of a saline suspension of *Listerella* (density McFarland Nephelometer Tube 5). For 2 days after inoculation intermittent blinking was observed, but no further evidence

of conjunctivitis or keratitis appeared. The pigs exhibited slight temperature reactions, one for 2 days and the other for 3 days.

One pig died 11 days after inoculation and *Listerella* was recovered from the medulla. The other pig died 32 days after exposure, but bacteriologic cultures failed to reveal *Listerella* in the heart blood or medulla. Since *Listerella* ordinarily produces only a local infection when instilled into the eye, the death of one pig from systemic infection after conjunctival exposure is of particular interest.

Cats

Pirie (1927) found that feeding or subcutaneous inoculation of cats with a gerbille *Listerella* strain produced no effect. The ovine *Listerella* Strain 10957, employed in the experiments on sheep and cattle, was used in the study of listerellosis in cats at the Illinois Station.

Intracerebral inoculation. One cat was inoculated intracerebrally with .1 cc. of a saline suspension of *Listerella* (density 1-1000 McFarland Nephelometer Tube 1). Its temperature had risen to 105°

FIG. 12.—CAT AFFECTED WITH CONJUNCTIVITIS AND KERATITIS
This cat was intravenously exposed to *Listerella* 12 days before this picture was taken. Note the drooped ear.

F. the next day, and dullness, weakness, and inappetence were present. The following day ataxia and weakness were observed, and the temperature was 105.8° F. In the course of the next week, the temperature gradually returned to normal and the animal recovered.

Intravenous inoculation. One cat was given intravenously 2 cc. of a saline suspension of *Listerella* (density McFarland Nephelometer Tube 1). The next day its temperature was 104.8° F., and it remained high for 5 days more. The day after inoculation the animal exhibited dullness, weakness, inappetence, and it weaved in walking. Its condition became steadily worse. Ten days after exposure it could not walk and paralysis of the hind legs was observed 2 days later. At the time that the paralysis appeared, keratitis of the left eye and a moderate exudate from the right eye were present (Fig. 12). Death finally occurred 17 days after treatment. At autopsy necrotic lesions similar to those in affected chickens were found in the myocardium of the cat. The keratitis and conjunctivitis were still present but no other gross lesions were found. Bacteriologic cultures were made from the cerebrum, medulla, lumbar cord, heart blood, liver, spleen, kidney, and aqueous humor. *Listerella* was isolated from the kidney and aqueous humor.

Dogs

Pirie (1927) found that the feeding or the subcutaneous or intraperitoneal inoculation of the gerbille strain of *Listerella* produced no effect on dogs.

At the Illinois Station the ovine *Listerella* Strain 10957 was employed for the study of experimental listerellosis in dogs.

Intravenous inoculation. A seven-week-old collie puppy was given intravenously .1 cc. of a saline suspension of *Listerella* (density McFarland Nephelometer Tube 10). The following day its temperature had risen to 105° F., and the puppy was less active than usual and had little appetite. During the next few days the temperature returned to normal and no further symptoms developed.

Supraconjunctival inoculation. Another seven-week-old collie puppy was given .1 cc. of a similar *Listerella* suspension by instillation into the eye. A second treatment was administered 2 days later. Neither temperature reaction nor ocular symptoms resulted.

Chickens

Seastone (1935) caused death in chickens by intravenous inoculation with chicken strains of *Listerella*. Paterson (1937) reported that inoculation of chickens with strains of *Listerella* isolated from chickens resulted in death. He later (1940B) found that death could be pro-

TABLE 17.—RESULTS OF INTRACEREBRAL INOCULATION OF 10-WEEK-OLD CHICKENS WITH LISTERELLA

(.1 cc. of a saline suspension with the density of McFarland Nephelometer Tube 1 was used and diluted as indicated)

Chicken No.	Dilution of inoculum	Days until symptoms appeared	Days until death	*Listerella* recovered (+) or not recovered (−)			
				Heart blood	Liver	Brain	Bone marrow
1031	1-10	1	1	−	..	+	..
1041	1-100	..	2	−	..	+	..
917	1-100	1	2[a]	+	..	+	+
1033	1-1,000	2	3	−	−	+	−
956	1-1,000	2	2[a]	+	..	+	+
1085	1-10,000	1	2	−	−	+	+
699	1-10,000	..	(survived)				
1007	1-20,000	2	2[a]	..	..	..	..
597	1-50,000	1	2	+	..	+	..
707	1-100,000	..	(survived)				
696	1-1,000,000	2	4	+	..	+	+

[a]Killed when moribund.

duced regularly by inoculation of chick embryos and studied the pathology of the disease in the embryo and its membranes. Cole (1941) caused monocytosis and leucocytosis followed by recovery in 4 chickens by giving them an intraperitoneal inoculation. Schwarte and Biester (1942) reported death in 2 out of 3 hens inoculated intracranially with a bovine strain of *Listerella;* they were unable to produce a reaction in 3 other hens inoculated intravenously.

In experiments with chickens at the Illinois Station the ovine *Listerella* Strain 10957 was used.

Intracerebral inoculation. A saline suspension of *Listerella* was administered intracerebrally to each of 11 ten-week-old chickens. A suspension equivalent in density to the McFarland Nephelometer Tube 1 was used after being diluted from 10 to a million times. A dose of as little as .1 cc. of a McFarland Tube 1 suspension diluted a million times was sufficient to cause death in one bird (Table 17). *Listerella* was isolated from the heart blood, brain, and bone marrow of this bird. The birds which died showed symptoms of a disturbance of the central nervous system. All birds showed weakness, dullness, and sleepiness and ataxia was observed in some. Some birds fell over backward when set upon their feet. Usually they lapsed into coma before death. A bird inoculated with a dilution of 1-10,000 and one with a dilution of 1-100,000 remained healthy and both were finally released. The other 9 birds died between 1 and 4 days after inoculation. *Listerella* was isolated from the heart blood of 4 out of 8 birds cultured, from the brain of all 8 cultured, and from the bone marrow

TABLE 18.—RESULTS OF INTRAVENOUS INOCULATION OF 10-WEEK-OLD CHICKENS WITH LISTERELLA

Chicken No.	Density of inoculum (McFarland Nephelometer No.)	Dosage in cc.	Days until symptoms appeared	Days until death	*Listerella* recovered (+) or not recovered (−)			
					Heart blood	Liver	Brain	Bone marrow
920	1	.2	1	(survived)				
605	1	.5	1	11	+	..	−	+
931	1	.5	1	2	+	..	+	+
827	1	.5	..	(survived)				
374	1	.5	..	(survived)				
636	1	1.0	1	2	+	..	+	+
1025	5	.5	3	5	..	+	+	+
566	5	1.0	3	11	+	..	+	+
1042	5	2.0	3	3	..	+	+	+

of 4 out of 5 cultured. No *Listerella* was recovered from the 2 livers cultured.

Intravenous inoculation. Varying doses of a saline suspension of *Listerella* were administered intravenously to 9 ten-week-old chickens. Six birds died; 3 birds which received relatively small doses survived (Table 18). One of the surviving chickens became weak and dull the day after inoculation. It remained in this condition for 6 days before it recovered. Symptoms of weakness and dullness were observed in the chickens which died, and diffuse necrosis of the myocardium was found at autopsy. *Listerella* was recovered from the heart blood of the 4 birds cultured, from the liver of the 2 birds cultured, from the brain of 5 out of 6 birds cultured, and from the bone marrow of the 6 birds cultured.

TABLE 19.—RESULTS OF SUBCUTANEOUS INOCULATION OF 10-WEEK-OLD CHICKENS WITH LISTERELLA

Chicken No.	Density of inoculum (McFarland Nephelometer Tube No.)	Dosage in cc.	Days until death
614	1	1.0	4
1006	5	.2	(survived)
1020	5	.2	(survived)
1055	5	.4	(survived)
1088	5	.4	(survived)
1034	5	.6	(survived)
1015	5	.6	(survived)
1097	5	1.0	(survived)
1066	5	1.0	(survived)
633	10	.2	24
630	10	.5	(survived)
677	10	1.0	(survived)

Subcutaneous inoculation. Varying doses of a saline suspension of *Listerella* were given subcutaneously to 12 ten-week-old chickens (Table 19). *Listerella* was not isolated from the 2 birds which died. All birds except Nos. 630 and 677 showed symptoms of coryza, and it is probable that this disease rather than the injection of *Listerella* was responsible for the 2 deaths.

Supraconjunctival inoculation. Varying amounts of a saline suspension of *Listerella* were instilled into the eyes of 8 ten-week-old chickens. Four birds which received .05 cc. of a McFarland Nephelometer Tube 10 suspension showed no symptoms. One bird which had received this dosage and 2 other chickens which had received .1 cc. of the same suspension exhibited a transient conjunctivitis 6 days after treatment.

Feeding Listerella. Doses of 1 cc. of a saline suspension (density McFarland Nephelometer Tube 10) were fed daily for a week to 5 ten-week-old chickens. No symptoms were observed.

Discussion of Results With Experimental Listerellosis

Pathogenicities. Intracerebral inoculation with *Listerella* caused death in the sheep, cattle, horse, and chicken in experiments carried out at the Illinois Station. A cat inoculated by this route became very ill but recovered.

Contrary to the reports of Gill (1933, 1937), Paterson (1940D), Olafson (1940), and Schwarte and Biester (1942), which stated that there was little or no reaction in sheep after intravenous inoculation with *Listerella,* sheep at the Illinois Station died following inoculation by this route. Intravenous inoculation also caused death in one pig, several chickens, and in a cat that had developed conjunctivitis and keratitis. In cattle, horses, and another pig, intravenous inoculation caused illness but it was followed by recovery. Only slight indisposition was produced in a puppy after inoculation by this route.

Subcutaneous inoculation with *Listerella* had no effect upon chickens nor did feeding of the culture to these birds.

Intragastric exposure of a sheep and a horse was followed by temporary illness, but it had no result in a steer and another horse.

A characteristic action of *Listerella* inoculated supraconjunctivally into rabbits and guinea pigs was to produce conjunctivitis and keratitis. Similar lesions were induced in lambs exposed in the same way, but only a mild, transitory conjunctivitis developed in a steer and a horse following similar treatment. Upon recovery these animals were not affected by repeated conjunctival inoculation with *Listerella,* a fact which would suggest that some protective influence had been developed. No ocular symptom other than blepharism was observed in a young pig exposed to *Listerella* by the conjunctival route, but the pig died in 11

days and *Listerella* was recovered from the medulla upon culture. Marked conjunctivitis and keratitis appeared in a cat which had been inoculated intravenously with *Listerella.* Supraconjunctival exposure of a puppy produced no visible effect. A mild, transient conjunctivitis was observed in 3 out of 8 chickens inoculated supraconjunctivally with *Listerella.* It is thus seen that, in many species of animals, conjunctivitis follows instillation of *Listerella* into the eye.

Hematologic changes. Murray, Webb, and Swann (1926) gave the specific name *monocytogenes* to the *Listerella* strains which they isolated from rabbits and guinea pigs because of the marked monocytosis which the organism produced in these animals. Further extensive work has been done upon the monocytic reaction in laboratory animals exposed to listerellosis. The mononucleosis was confirmed in rabbits by Seastone (1935), Morris and Julianelle (1935), Webb and Barber (1937), Pons and Julianelle (1939), and Julianelle (1940); and in guinea pigs by Seastone (1935), Morris and Julianelle (1935), and Pons and Julianelle (1939). The monocytic reaction was observed in mice by Pons and Julianelle (1939) and in chickens by Seastone (1935). Slabospits'kii (1938) reported that monocytes and neutrophiles increased and megaloblasts appeared in the blood of infected rabbits. Julianelle and Pons (1939A) noted that the exudate in the eyes of supraconjunctivally exposed rabbits, guinea pigs, and rats contained a large number of monocytes.[1]

In one case in man, Gibson found 16 percent monocytes in the meningeal pus. Nyfeldt (1929, 1932) and Schmidt and Nyfeldt (1938) isolated *Listerella* from a number of cases of infectious mononucleosis. Pons and Julianelle (1939) isolated the organism from a girl suffering with infectious mononucleosis; her differential count revealed 40 percent monocytes. The etiologic relationship of *Listerella* to infectious mononucleosis, however, is far from clear, according to Julianelle (1940) and it is at present considered improbable that this organism is the cause of the disease.

There is little evidence that a marked circulating monocytosis is characteristic of listerellosis in ruminants. Gill (1931) reported an increase in neutrophiles and a decrease in lymphocytes in field cases of ovine listerellosis. Jungherr (1937) found a normal differential count in an experimentally infected lamb. Muth and Morrill (1942) found 5 percent monocytes in one naturally affected lamb on which they made a differential count. In observations at the Illinois Station, leucopenia

[1]The monocytic reaction in the small laboratory animals is so constant and characteristic that it has been utilized by hematologists in investigations on the origin and nature of the monocyte. For a discussion of the subject see Witts and Webb (1927), Bloom (1928), Lang (1928), Bianchi (1930), Nyfeldt (1932), Rezzesi (1933), Wallbach (1934), Anton (1934), Levi and Penati (1934, 1935), Penati and Levi (1935A, 1935B), and Conway (1938, 1939).

was noted in two field cases of listerellosis, and a high percentage of neutrophiles in another.

In experimentally exposed sheep, leucocytosis followed intracerebral, intravenous, and intragastric inoculations but not supraconjunctival exposure. An increase in neutrophiles and a decrease in lymphocytes followed intracerebral and intravenous inoculation; whereas after intragastric exposure, the lymphocytes increased and the neutrophiles decreased. After supraconjunctival exposure the monocytes increased slightly to 9 percent in one sheep, but the monocyte level remained unchanged in the other animals.

In cattle experimentally exposed to listerellosis no significant changes in either the total or differential leucocyte counts were observed in one animal after intragastric exposure or after intravenous exposure. In a pregnant heifer exposed intravenously, however, a leucopenia developed, accompanied by a marked decrease in the percentage of neutrophiles and a temporary slight monocytosis (13 percent monocytes). The monocytosis was not caused by an increase in the number of monocytes but rather by a decrease in the number of neutrophiles and lymphocytes. It was therefore purely relative and not actual. After supraconjunctival exposure of one animal, a slight relative monocytosis (12 percent monocytes) was noted.

In experimentally inoculated horses, leucocytosis characterized by an increase in neutrophiles followed intracerebral and intravenous inoculation. One horse inoculated intravenously exhibited a very slight monocytosis (9 percent). One horse fed *Listerella* by stomach tube exhibited a leucopenia characterized by a decrease in neutrophiles, but no change was observed in the blood of another horse similarly exposed, as well as in another inoculated supraconjunctivally.

In all these cases in which an increase in percentage of monocytes was observed, the increase was not very large and lasted only a short time. Certainly the marked monocytosis which occurs in rodents—that observed by Julianelle (1940), for example, in which he reports 29 percent and 51 percent monocytes for 2 rabbits—was not observed in ruminants at the Illinois Station by Graham, Dunlap, and Levine (1940).

Both leucopenia and leucocytosis have been observed in natural cases of listerellosis. This apparent contradiction may be explained in part by the fact that the blood counts were made in different stages of the disease. In experimentally infected animals leucocytosis usually followed inoculation. In some cases the leucocytosis was then followed by leucopenia; in others it was not. In still other experimental animals leucopenia was observed after inoculation and it was not preceded by leucocytosis. Differences in route of inoculation, in amount of inoculum, and in individual resistance might account for the inconsistency. This question deserves further study.

HISTOPATHOLOGY

Descriptions of the histopathologic features of listerellosis presented by independent investigators provide a fairly clear conception of the minute morphological changes encountered in the natural and experimental infection. These changes have proved valuable in confirming bacteriologic diagnoses of natural outbreaks and in suggesting the nature of the disease when bacteriologic methods failed or could not be carried out.

Rabbit. In the rabbit the disease has been characterized by a circulating monocytosis and necrotic foci in the liver. Murray, Webb, and Swann (1926) described the disease as a large mononuclear leucocytosis but did not mention any histopathologic changes. Paterson (1940C) described the liver in a pregnant rabbit affected with listerellosis as being enlarged, friable, and studded with grayish white, pinhead foci; the foci exhibited necrosis without cellular infiltration. The uterus in this particular animal showed an intense suppurative endometritis and slight degenerative changes in the myometrium. Large numbers of bacteria were noted at the junction of the fetal membrane and the uterine mucosa, as well as thruout the tissues of the fetuses. From the reports of Gill (1931), Burn (1934), Schultz *et al.* (1934), and Seastone (1935), it is evident that experimental infection of rabbits by the intravenous route commonly may result in necrotic foci in the liver and myocardium and occasionally in other organs. These foci are often invaded by mononuclear and polymorphonuclear leucocytes (Fig. 13). The brain and meninges often show considerable congestion and infiltration with leucocytes, particularly with neutrophiles, lymphocytes, and monocytes.

Definite cases of the disease in rabbits failed to follow the administration of *Listerella* by the following routes: intramuscular, intraperitoneal, Gill (1933) and Burn (1934); subcutaneous and oral (given with a stomach tube), Gill (1933). Nasal insufflation resulted only in a nasal catarrh, according to the findings of Gill (1933). Jones and Little (1934) and Webb and Barber (1937) found that intracerebral inoculation was followed by meningitis or meningo-encephalitis, especially near the site of inoculation, and that sometimes invasion of the circulation and liver lesions followed. Webb and Barber described the cells of the meningeal exudate as mononuclear and polymorphonuclear with a much higher proportion of neutrophiles than were seen in other locations. Supraconjunctival inoculation produced changes in rabbits resembling, in gross at least, those which follow like inoculation of the guinea pig. Conjunctivitis has also been observed at the Illinois Station in the uninoculated eye of a rabbit which had contact with other artificially infected animals, thus suggesting the possibility of contact infection by way of the conjunctiva.

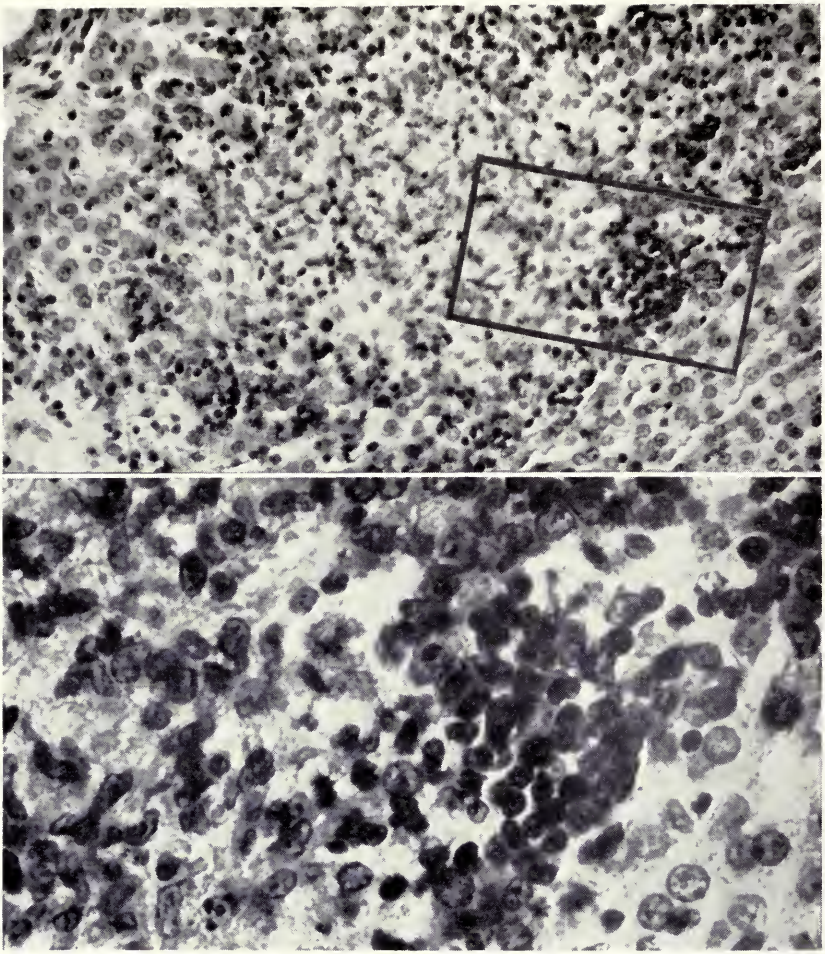

FIG. 13.—LIVER FROM A RABBIT WITH EXPERIMENTAL LISTERELLOSIS

Focal necrosis of hepatic cells has taken place and lymphocytes and mononuclear cells have infiltrated. Section *above* is magnified 265×; marked area is shown below magnified 750×.

(All photomicrographs, Figs. 13-21, were made from sections stained with hematoxylin and eosin.)

Guinea pig. Jones and Little (1934) reported that intracerebral inoculation caused a fatal meningitis in guinea pigs. Results similar to those in the rabbits were reported by Schultz *et al.* (1934) and Burn (1934) as following intravenous or intracranial inoculation and also by Seastone (1935) as taking place after intravenous inoculation. At the

Illinois Station supraconjunctival exposure resulted in a catarrhal to follicular conjunctivitis and a keratitis which occasionally terminated in ulceration. In sections the conjunctiva showed degenerative changes in the epithelium in the form of vacuolation and loss of architecture with varying degrees of sloughing. The conjunctiva was infiltrated with

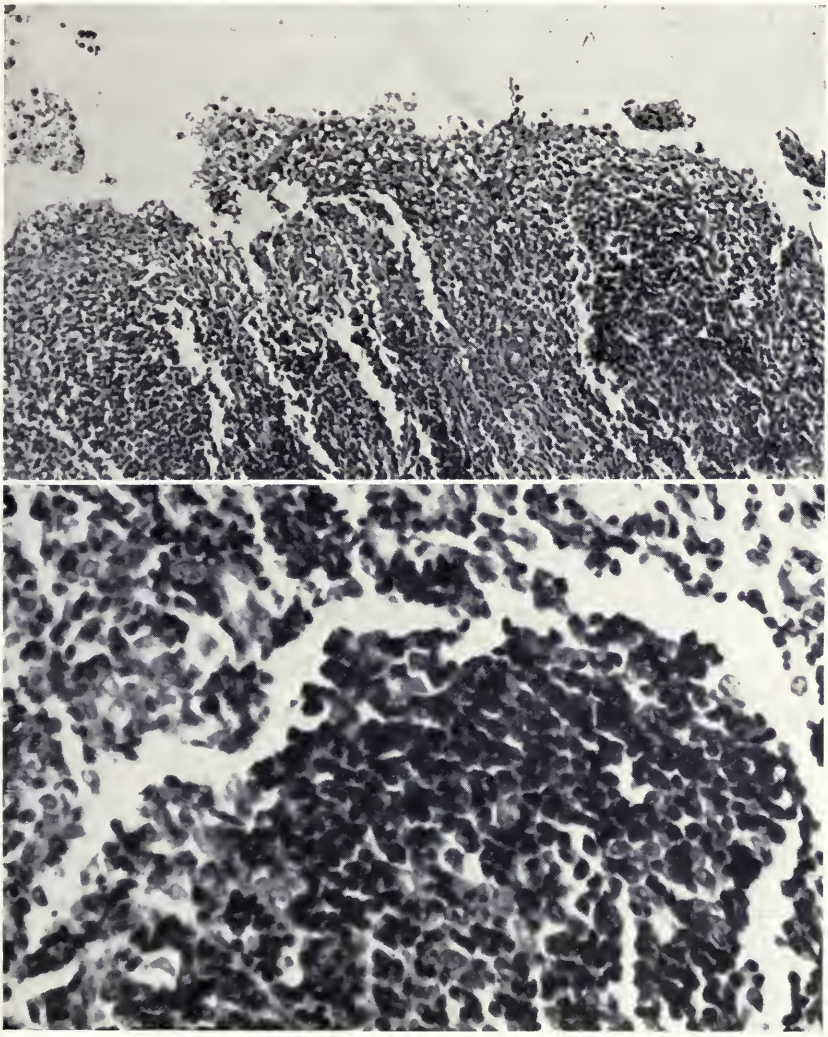

FIG. 14.—CONJUNCTIVA OF GUINEA PIG WITH EXPERIMENTAL LISTERELLOSIS
Note follicle-like accumulations of lymphocytes and mononuclear cells. Section *above* is magnified 225×; area *below* is from a similar section magnified 415×.

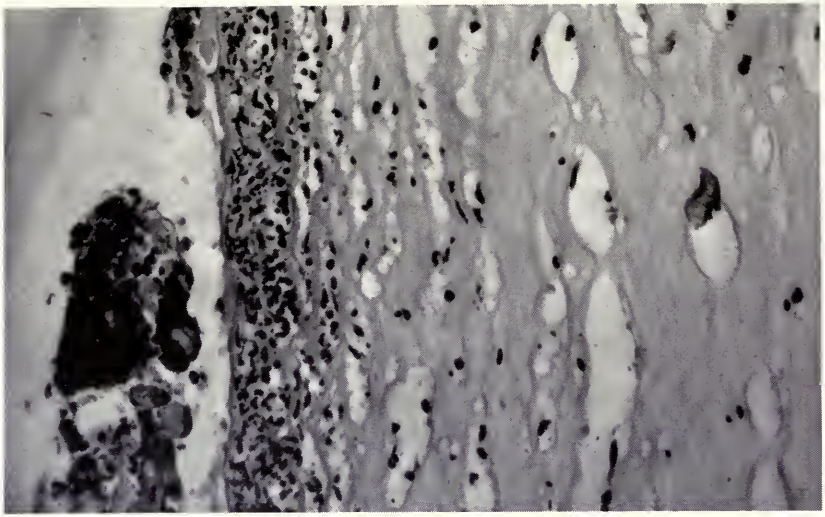

FIG. 15.—CORNEA OF GUINEA PIG WITH EXPERIMENTAL LISTERELLOSIS
Neutrophiles have infiltrated. They are particularly noticeable in the vicinity of Bowman's membrane and in the surface exudate. Magnified 265×.

lymphocytes, plasma cells, neutrophiles, and some large mononuclear cells. Deeper in the conjunctiva were several follicle-like accumulations of cells of the lymphocytic series with a few larger mononuclear cells intermixed (Fig. 14). In the cornea there was extensive necrosis and desquamation of the epithelium; leucocytes, predominantly neutrophiles, had infiltrated, and capillaries containing some erythrocytes were pushing down into the substantia propria. The infiltrating neutrophiles found in the interlamellar spaces were most numerous in the vicinity of Bowman's membrane (Fig. 15). These findings are in general accord with the more detailed description of the process by Julianelle and Moore (1942).

Mice. Listerellosis is not reported as a naturally acquired disease of mice. Webb and Barber (1937) described in some detail their findings in a series of intraperitoneal inoculations made on 60 white mice. They report that the peritoneal exudate, when present, was present in a small amount and was very cellular, with mononuclear cells predominating but neutrophiles always present. Multiple grayish-yellow foci of necrosis were seen in the livers of nearly all animals. Frequently they were more numerous just beneath the capsule. The centers in each focus consisted of a collection of mononuclear cells in a coarse reticulum that had a marginal zone of nuclear debris containing some neutrophiles and lymphocytes. The organisms were usually plentiful in the periphery of each focus. In animals surviving less than

12 hours, necrosis had not yet occurred, and the lesion consisted of clumps of bacteria with a few mononuclear cells around them. Liver cells in the vicinity of the necrotic foci frequently had undergone fatty changes. Foci of necrosis were sometimes found in the mesenteric lymph nodes, kidneys, and suprarenal glands also. The presence of clumps of organisms under the capsules of these organs suggests spread by direct extension. Organisms were numerous in the spleen; these were often present in large masses just beneath the capsule, also suggesting invasion from the peritoneal cavity. Focal lesions were found in areas adjacent to the Malpighian corpuscles. These lesions consisted chiefly of mononuclear cells and large, pale cells of the reticulo-endothelial system of the splenic pulp, among which early nuclear degeneration was described.

In the lungs, the only lesions described by Webb and Barber were congestion, occasional hemorrhage, and some atelectasis. Intracranial and intravenous inoculation evidently produced some of these changes also, and intracranial inoculation, in addition, resulted in a meningeal exudate near the site. The cells involved were mononuclear and polymorphonuclear. Phagocytosis of many organisms was noted and hemorrhage was present.

Wright and MacGregor (1939) reported that intraperitoneal inoculation of mice produced "typical" liver lesions. Cole (1941) found focal necrosis of the liver and spleen in 2 mice that died 3 days after an intraperitoneal inoculation with a strain of *Listerella* isolated during an outbreak in chickens.

Gerbille. Pirie (1927) described the microscopic changes produced by listerellosis in the gerbille. In the spleen, necrosis of the pulp was observed, together with marked karyorrhexis and the presence of large numbers of the causative organism. The liver showed multiple areas of necrosis, which were commonly but not always preceded by fatty changes. Pirie likewise observed little leucocytic reaction in the vicinity of the necrotic foci, but did observe in the gerbille considerable proliferation of capillary endothelial cells and the presence of an occasional giant cell at the periphery of the necrotic focus. In some cases early bronchopneumonia was observed. Kidney and heart showed only cloudy swelling. In the small intestine, ulcers characterized by V-shaped necrotic areas extending thru to the peritoneum were observed in some cases. Outside the clearly necrotic areas were zones of congested vessels, some karyorrhexis, and abundant bacteria. These lesions were reproduced in the gerbille by putting out bait into which *Listerella* cultures had been incorporated.

Monkey. Schultz *et al.* (1934) found a strain of *Listerella* isolated from a case of human meningo-encephalitis to be pathogenic for rhesus monkeys after intravenous or intracerebral inoculation. The lesions were not described as differing from those found in mice, guinea pigs,

or rabbits. Burn (1934) also stated that the organism isolated from infants was to some degree pathogenic for young monkeys.

Chickens. No detailed results of histopathologic studies on naturally occurring listerellosis in chickens are available. The condition, as previously noted, is considered to be a septicemia often characterized by focal but massive areas of necrosis in the heart muscle.

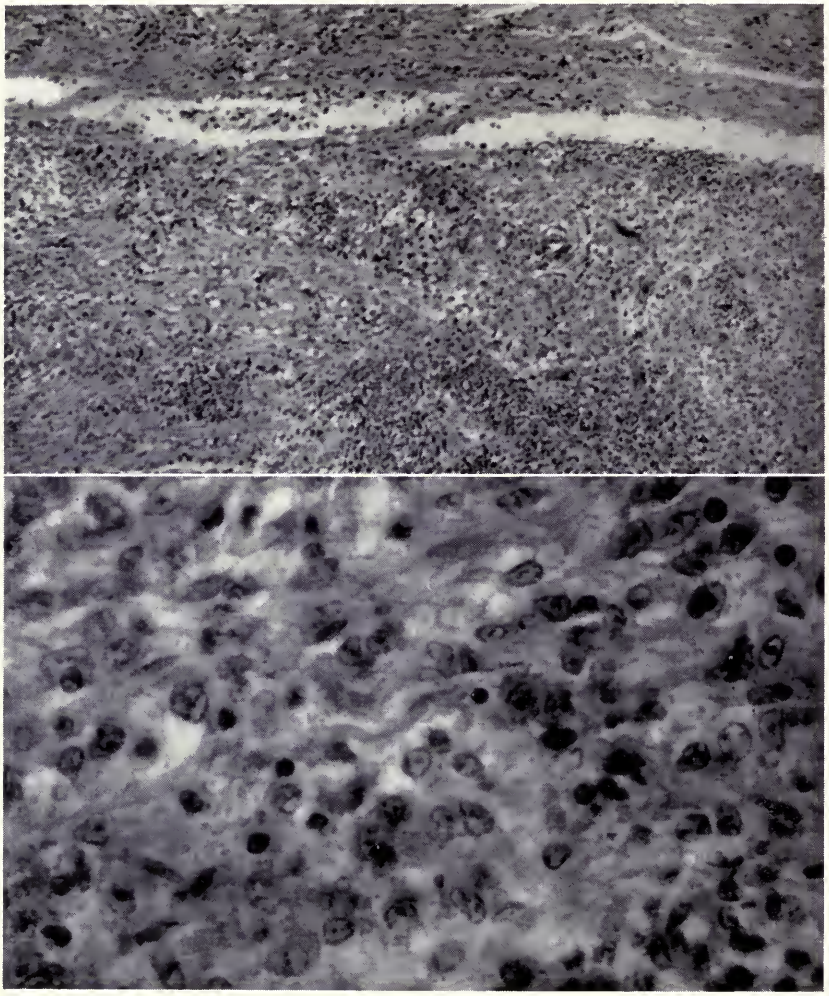

FIG. 16.—MYOCARDIUM OF CHICK INOCULATED INTRAVENOUSLY WITH *Listerella*
Note disappearance of myocardial fibers and infiltration with mononuclear and lymphoid cells. Section *above* is magnified 145 ×; area of infiltration *below* is from same section magnified 750×.

Cole (1941) in 2 out of 8 cases observed in addition small ulcers in the ileum and ceca.

In cases of listerellosis experimentally produced in chickens, Seastone (1935) observed that intravenous inoculation resulted in a massive necrosis of the myocardium, pericarditis, and congestion of liver and spleen, but only slight meningitis or perivascular infiltrations in the central nervous system. Graham, Hester, and Levine (1940A) also observed the myocardial necrosis as a result of intravenous inoculations. The necrotic areas were quite heavily infiltrated with a cellular exudate in which mononuclears predominated (Fig. 16). Cole (1941) observed a relative monocytosis following intraperitoneal inoculation.

By intraallantoic inoculation of a standardized dose, Paterson (1940B) was able to produce lesions in both the developing chick embryo and the chorio-allantoic membrane and the embryo died within 72 to 96 hours. The lesions in the chorio-allantoic membrane were noted as early as 9 hours after inoculation, at which time small patchy areas of endodermal cells appeared swollen and granular. Swelling of mesodermal cells also occurred simultaneously. Thirty hours after inoculation small outgrowths, 2 to 4 cells thick, were present, and the membrane appeared slightly hazy to the naked eye. By 36 hours after inoculation numerous tiny white foci were observed over the entire chorio-allantoic membrane. Leucocytic invasion occurred, and endodermal lesions became more extensive. The ectoderm remained unchanged; bacteria were not observed in it altho numerous elsewhere.

Within the embryo Paterson observed no lesions until about 18 hours after inoculation, at which time cloudy swelling and vacuolation of the hepatic cells were observed. At about 48 hours after inoculation pin-point to pinhead foci of necrosis appeared; these enlarged but did not greatly increase in number. Finally there was infiltration of the parenchyma by mononuclear cells. After 36 hours the heart also showed small necrotic foci, which became very extensive. Late in the infection bacteria were observed in the meninges and the cancellous spaces of the cranial bones. Occasional foci of small round cells and a few heterophiles (homologues of the neutrophiles of other species) were seen adjacent to the affected sites. At death small foci of necrosis were noted in the midbrain and cerebrum.

Sheep. Among the six cases in sheep observed by Gill (1931), the following aggregate lesions were described, the distribution and degree varying in different animals: edema of meninges, purulent foci in the pia mater and striated nucleus, perivascular cuffing, and foci of cells not visibly associated with the vessels in various parts of the brain. Specifically mentioned areas of involvement were the meninges, hippocampus, striated nucleus, corpus striatum, corpora quadrigemina, and cerebellum. The infiltrating cells were described as round cells and neutrophiles, with the neutrophiles predominating.

Doyle (1932) gives an account of outbreaks of encephalitis in two flocks of sheep. The encephalitis was characterized by perivascular infiltration of cells, chiefly round or lymphoid cells but these were

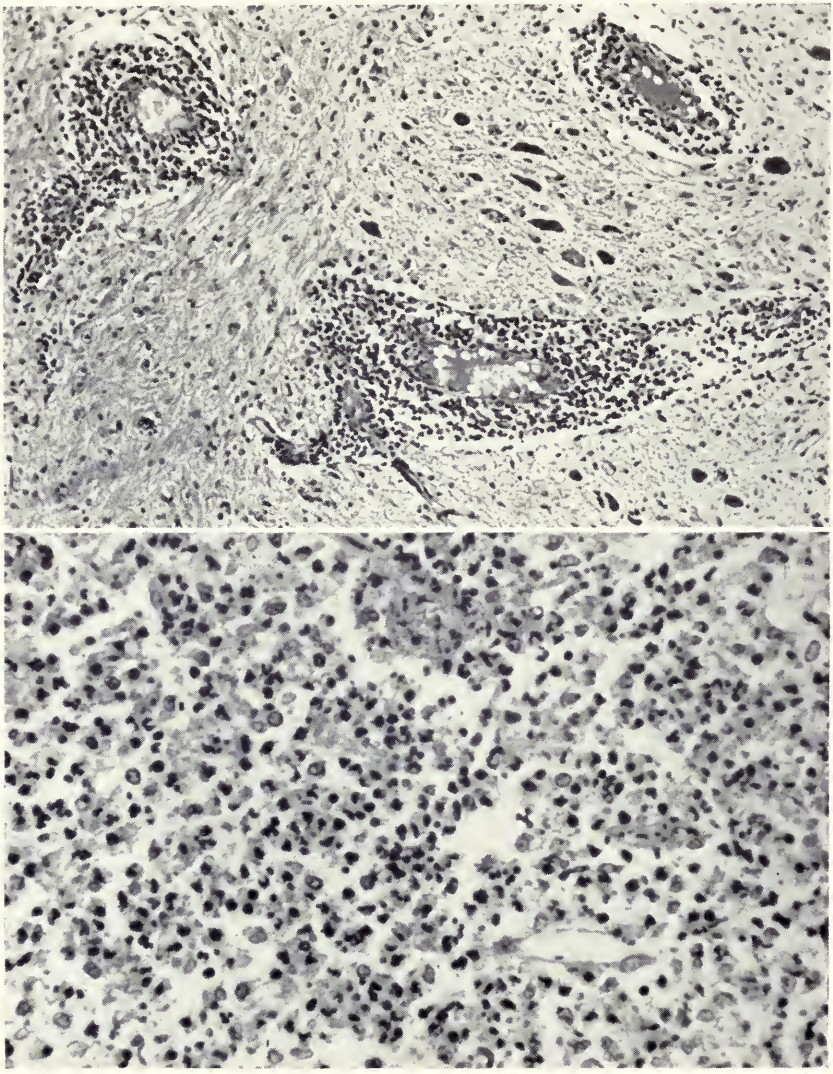

FIG. 17.—BRAIN STEM FROM A SHEEP NATURALLY INFECTED WITH LISTERELLOSIS

Perivascular infiltration with lymphoid and mononuclear cells and necrosis of neurons are shown *above* magnified 100×. *Below* is shown focal cellular reaction in which neutrophiles predominate (magnified 300×).

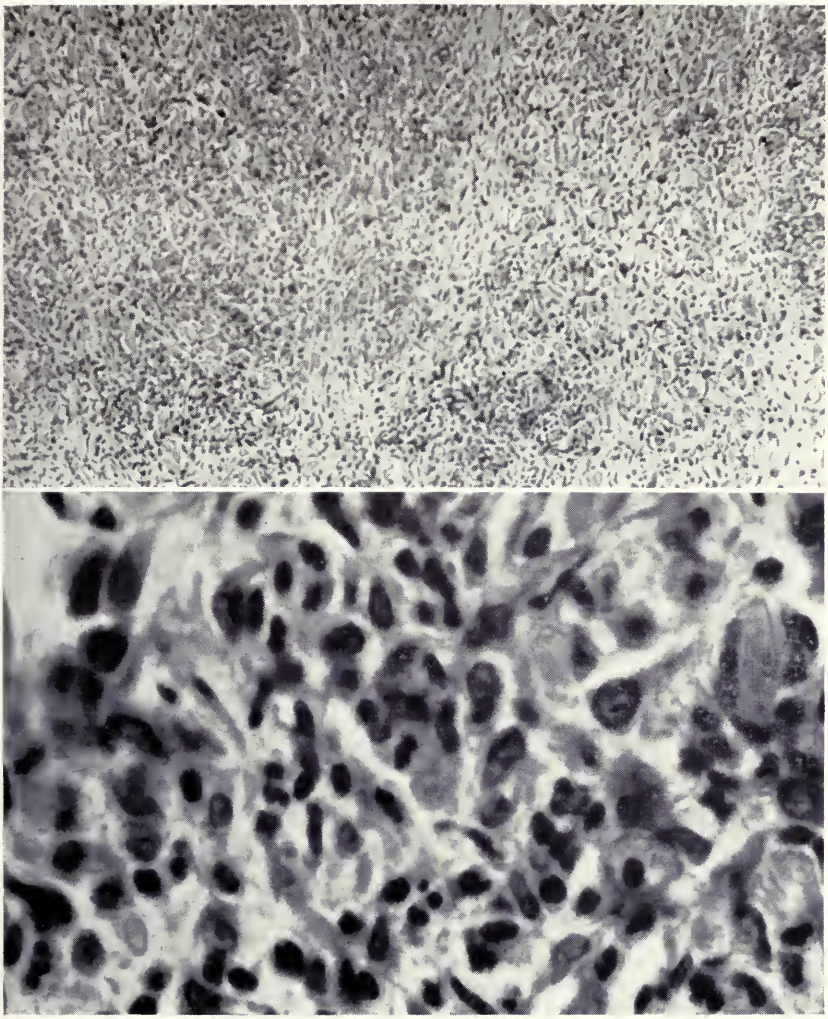

FIG. 18.—MYOCARDIUM OF SHEEP INOCULATED INTRAVENOUSLY WITH LISTERELLA

Note the extensive destruction of muscle fibers and infiltration with lymphoid and mononuclear cells. An occasional fibroblast shows evidence of activity. Magnified *above* 100×, *below* 750×.

mixed with a few neutrophiles and large mononuclear cells. Doyle also stated that there were "areas of essential brain tissue which showed well-marked degenerative changes." He found no cell inclusions. This encephalitic condition was not definitely identified with

Listerella by bacteriological examination but the description of it suggests that it might well have been.

Jungherr (1937) described five cases of an ovine encephalomyelitis associated with *Listerella* infection in which he found inflammatory changes in the medulla oblongata. These changes consisted of the appearance of foci of neutrophiles and the meningeal and perivascular infiltration of monocytes. Slight parenchymatous degeneration and fatty changes in the liver also occurred at the same time.

Graham, Dunlap, and Brandly (1938) reported two outbreaks in sheep, histopathologic studies of which revealed that there were present "polymorphonuclear and mononuclear foci in the stem and in the white matter of the cerebrum and cerebellum, together with perivascular cuffing with mononuclear cells and a mononuclear meningitis." Subsequent studies have indicated that characteristic lesions include: (1) perivascular infiltration of lymphocytes, large mononuclear cells, and neutrophiles, with lymphocytes predominating (Fig. 17); (2) focal infiltration of cells which may or may not show vascular relationship and in which neutrophiles predominate (Fig. 17). Degenerative changes in neurons and focal hemorrhage are less constant.

Biester and Schwarte (1939), in describing the condition, reported that "frozen sections prepared from the anterior cord, medulla, pons, and hippocampus revealed advanced perivascular cuffing, focal infiltrations, and accumulations of monocytes and polynuclear leucocytes with necrosis." They also observed a marked cerebellar meningitis.

The nature of lesions produced artificially in sheep depended upon the route of inoculation. Intracerebral inoculation resulted in meningoencephalitis, which began at the point of inoculation and histologically resembled the naturally acquired disease. Encephalitis may also follow intravenous inoculation, and Gill (1933) observed it following inoculation into the carotid artery and repeated drenching by way of the nostril. Following intravenous inoculation one case at the Illinois Station developed extensive areas of necrosis in the myocardium. These areas were well infiltrated with mononuclear and lymphoid cells (Fig. 18).

Paterson (1940D) found that 2 pregnant ewes inoculated with *Listerella* aborted. There was an intense inflammation at the junction of the fetal and maternal cotyledons and many bacteria were present in the area. One of the 2 aborting ewes also showed meningo-encephalitis. Twenty-one days after abortion both ewes showed normal involution, but some organisms were still demonstrable in the uterine submucosa.

Biester and Schwarte (1939) reported a "marked intracapillary glomerulonephritis" as an outstanding change in a case of listerellosis experimentally produced in a sheep.

Cattle. Mathews (1928) reported an encephalitis in feeder cattle which might have been an outbreak of listerellosis. The outstanding lesion was a perivascular infiltration of leucocytes, predominantly

lymphocytes and mononuclear leucocytes, in the medulla. Areas of degeneration accompanied by pronounced cellular reactions in many nerve tracts were described. At first the cellular reactions consisted mainly of large mononuclear cells with polymorphonuclear leucocytes becoming quite numerous in more advanced lesions. Ganglion cells adjacent to areas of degeneration were described as retrogressing and vacuolation of the cytoplasm was noted in many mononuclear cells. Since no bacteriological examination was reported, it cannot be said with certainty that the report represents an outbreak of listerellosis.

Jones and Little (1934) also described an encephalitis in cows which was quite possibly associated with *Listerella* infection. No gross changes were observed, but the stem, midbrain, and anterior spinal cord showed microscopic lesions. In the first stage minute foci of softening and a slight infiltration of neutrophiles were found. As the neutrophiles became more numerous, round cells appeared. In the later stages round cells predominated and there were well-defined perivascular infiltrations. Cortical and meningeal lesions were infrequent. Results of animal inoculations indicated that the disease was infectious or at least transmissible, but the specific causative agent was not determined.

Fincher (1935) described four cases of an encephalitic syndrome in cows, three of which were definitely associated with the presence of a Gram-positive rod-shaped organism. At the time the report was made, 2 cases which had been examined histopathologically were characterized by perivascular accumulations of lymphoid cells.

Graham, Dunlap, and Brandly (1938) and Graham (1939) in their brief reports on the disease in cattle did not discuss the histopathologic aspects. However, histopathologic findings made at the Illinois Station (Fig. 19) have been in accord with those of other investigators. No histopathologic studies were made in the case of the *Listerella* isolated from a premature bovine fetus by Graham, Hester, and Levine (1939).

Biester and Schwarte (1941B) reported the results of histopathologic studies on a recovered case in cattle. Lesions included advanced perivascular infiltration, fields of monocytic infiltration, hemorrhages, and perivascular edema in widely scattered parts of the brain and in the anterior spinal cord. Degenerative changes and glial cell increase were noted in the cerebral cortex, with some meningeal congestion over that portion. Changes in the anterior spinal cord, medullary portion of the cerebellum, pons, medulla, piriform lobe, and optic chiasma were well marked; whereas those changes in the olfactory bulb and tract, cerebral cortex, hippocampus, and medullary portion of the cerebrum were not so severe. The cerebellar cortex was negative. Schwarte and Biester (1942) reported that in two cases of the naturally acquired infection there was an advanced focal and perivascular infiltration of monocytes and neutrophiles in the brain. In cattle

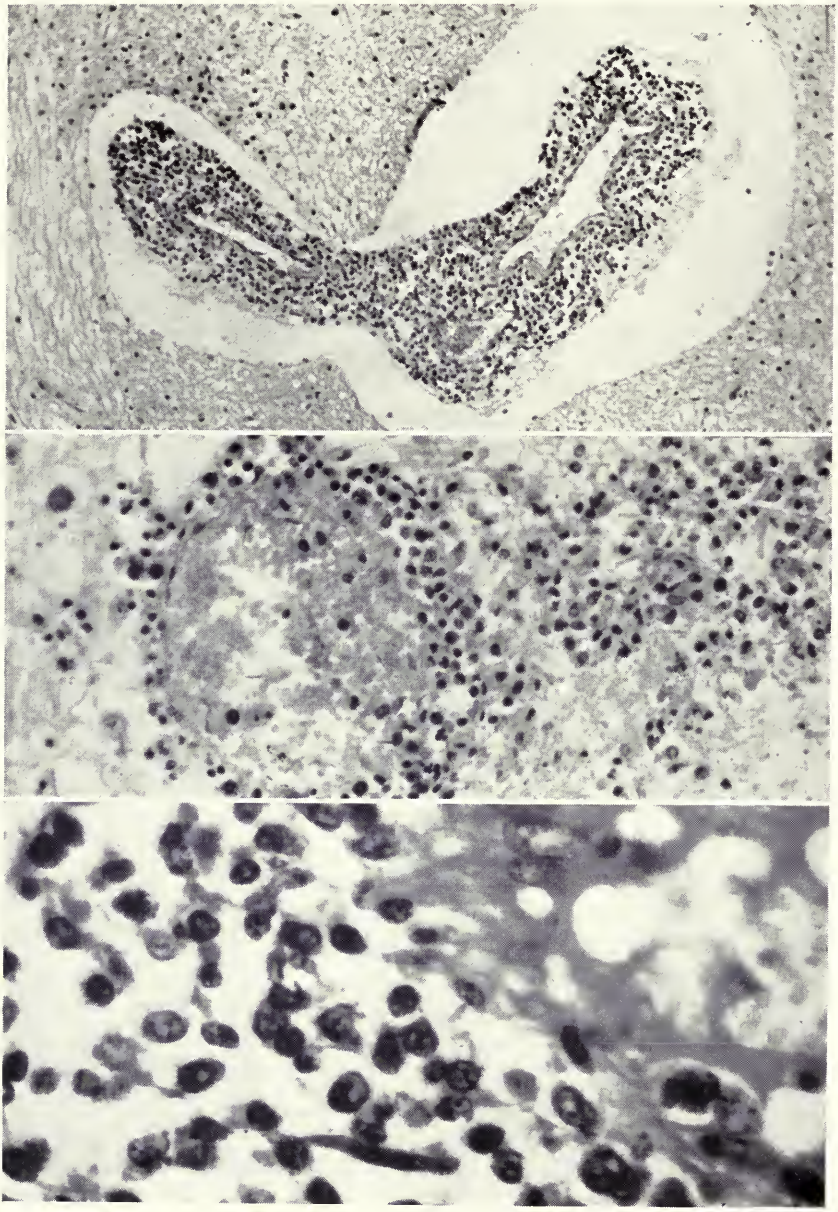

FIG. 19.—BOVINE BRAIN STEM FROM A NATURAL CASE OF LISTERELLOSIS
Perivascular infiltration of lymphoid and mononuclear cells is shown *above* magnified 100×, *center* 230×, and *below* 600×.

meningo-encephalitis may follow intracerebral inoculation with virulent *Listerella* cultures. When it does, it generally resembles the disease induced in sheep in like manner.

Goats. King (1940) reported the histopathologic changes in a primary encephalomyelitis in goats. In the central nervous system the lesions were restricted largely to the brain stem, especially the medulla and spinal cord. The typical lesion was described as a compact focal collection of cells varying up to one millimeter or more in diameter. The lengths of these foci were described as usually running rostrocaudally and ranging up to ten times the diameter. They usually were in close association with a blood vessel, either investing or in contact with it. The majority of the cells were mononuclear, but a few neutrophiles were present. Microglia and microglia-like cells, oligodendroglia and certain epithelioid cells were noted in the lesion. King also mentioned the presence of a syncytium in these areas. The vascular endothelium was usually swollen and proliferating, or degenerated with hyalinization. Changes in the media were sometimes pronounced, showing hyalinization and infiltration of the part with neutrophiles and mononuclear cells. King's description varied from that of some observers in that he did not note perivascular hemorrhage. Formation of small abscesses was seen rarely and then only in advanced cases. The spinal cord showed foci of demyelination. The meninges were commonly involved to a slight degree. Changes in nerve cells and the demonstrable presence of bacteria in the lesion were inconstant. One nodular lesion was noted in the spinal dura; however, the frequency of this lesion is not known, since the site usually was not examined. The livers were described as showing fatty changes or occasional focal necrosis. Glomerulitis was recorded in 3 cases. Experimentally induced infection in goats is not described.

Olafson (1940), in summarizing his studies on *Listerella* infection in sheep, cattle, and goats, considers the microscopic changes as marked and characteristic. Perivascular infiltration of cells is prominent. He reports the cell types present in order of their usual frequency as: lymphocytes, monocytes, neutrophiles, and eosinophiles. He considers the most characteristic lesion, however, to be the presence of foci of neutrophilic infiltration apparently independent of vascular relations (Fig. 17). These foci are said to contain the bacteria, while the perivascular infiltrations do not. Nerve cells show little evidence of damage. Olafson (1940) indicates that, in general, the brain stem from the anterior cervical cord to the thalamus is most severely affected; lesions in other parts of the brain and in the meninges are less constant and severe. These observations on the infection in sheep, cattle, and goats made by Olafson (1940) agree with those made on sheep and cattle at the Illinois Station.

Swine. Biester and Schwarte (1940), studying natural listerellosis in swine, found no gross lesions but did observe marked microscopic changes. They describe a meningitis characterized by marked monocytic infiltration and an encephalitis characterized by perivascular infiltrations, particularly in the region of the pons. There were numerous foci of monocytic infiltration in the brain stem, as well as a circu-

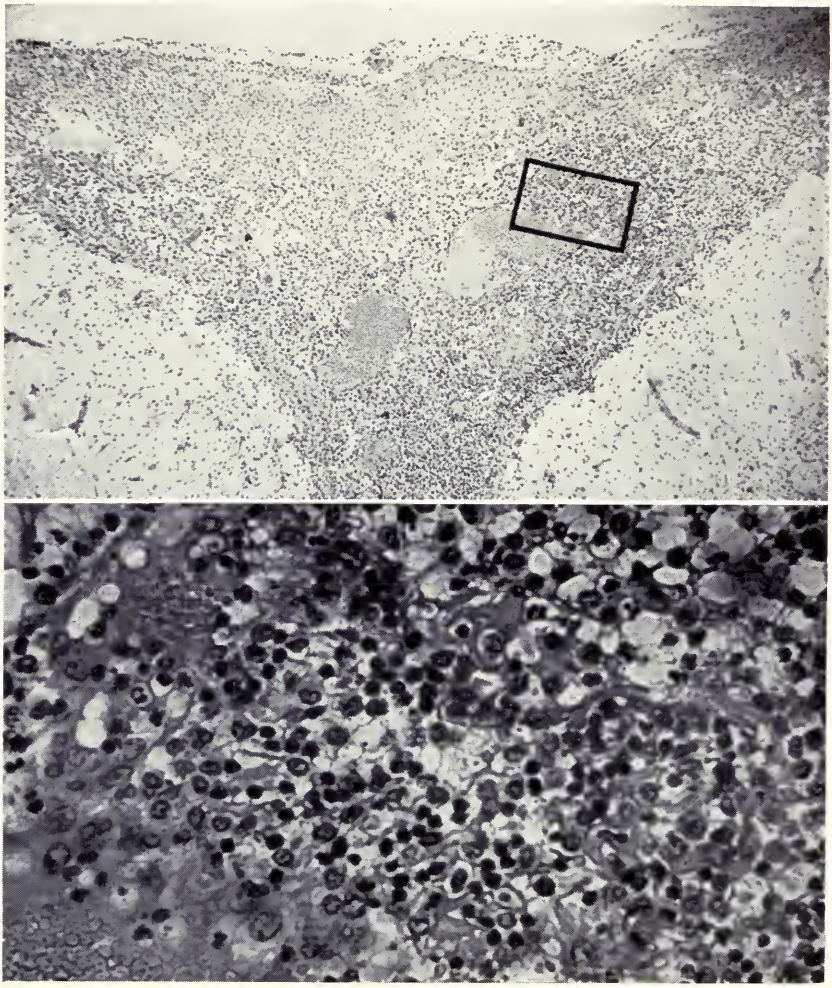

FIG. 20.—MENINGES FROM A HORSE INOCULATED INTRACRANIALLY WITH LISTERELLA

Exudate containing serum, fibrin, and cells is abundant. Section *above* is magnified 65×; marked area is shown *below* magnified 430×.

lating monocytosis. Polymorphonuclear leucocytes were present in considerable numbers in some microscopic fields.

Pigs generally develop a relatively severe meningo-encephalitis following intracranial inoculation and occasionally after intravenous, intramuscular, or even supraconjunctival exposure, according to Graham, Dunlap, and Levine (1940), and Biester and Schwarte (1940). Biester and Schwarte observed focal monocytic infiltrations in the kidney, frequently involving the glomeruli and adjacent areas.

Horses. A horse in which a fatal *Listerella* infection was induced by intracranial inoculation showed a large area of softening and hemorrhage with infiltration of neutrophiles at the point of inoculation. In the meninges, exudation of serum and cells occurred (Fig. 20). In some areas neutrophiles predominated, with fewer lymphocytes and mononuclears present. In other areas, and particularly deep in the sulci, mononuclear cells predominated (Fig. 21). The cerebral cortex showed occasional small foci of neutrophiles, usually just beneath the meninges (Fig. 21). The white matter, especially in the subependymal region, showed perivascular infiltration with lymphocytic elements and foci of neutrophiles and in some instances true suppuration. More extensive areas of neutrophilic infiltration with exudation of plasma proteins and hemorrhage were also noted. Staining characteristics of many neurons were altered in that bronzing occurred. Cuffing of vessels in the meninges of the anterior spinal cord with neutrophiles and a few lymphocytes took place. Considerable bronzing of neurons in the gray matter was noted here also. Occasional small foci of lymphocytes were seen in the liver and kidneys.

Man. Among the histopathologic changes described in human listerellosis by Burn (1936) and Wright and MacGregor (1939), the more prominent and constant changes were: suppurative leptomeningitis, focal areas of necrosis in the liver, focal pneumonia and bronchiolitis, and splenic engorgement. Also observed in the brain were hemorrhages and perivascular infiltration with neutrophiles, lymphocytes, and plasma cells.

Discussion of Histopathologic Findings

From the observations summarized, it is evident that the extent and degree of histopathologic changes produced by listerellosis vary somewhat in different species of animals. In the experimental disease the nature of the histopathologic changes depends to a greater or lesser extent upon the route of inoculation. Virulence of the organism may also be a factor, as pointed out by Schwarte and Biester (1942). When the microscopic lesions are present, they follow a rather constant pattern according to the organ affected.

Lesions produced in cases of listerellosis are largely limited to the

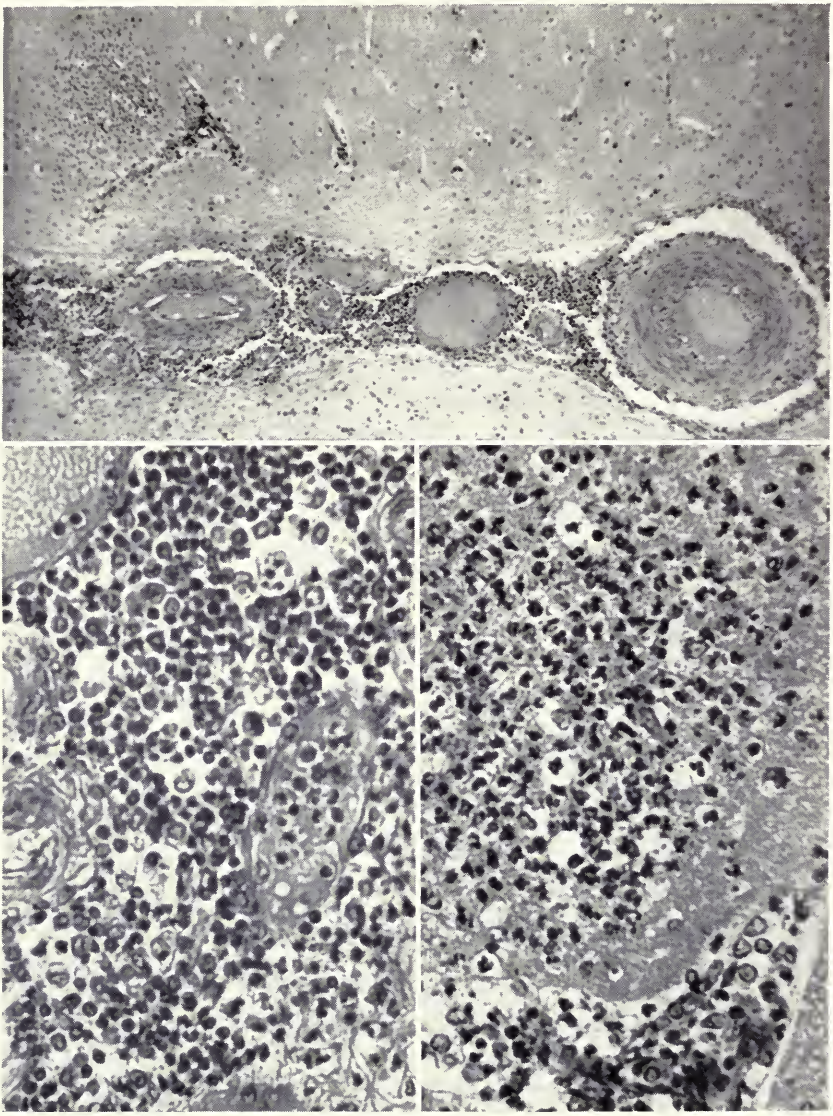

FIG. 21.—CEREBRUM FROM A HORSE INOCULATED INTRACRANIALLY WITH LISTERELLA

Note focus of neutrophilic infiltration in the cortex (magnified *above* 85× and to the right *below* 385×) and lymphoid and mononuclear infiltration in a sulcus (magnified *above* 85× and to the left *below* 385×).

central nervous system in the natural infection among ruminants, the form of the disease which affects the livestock industry most. In the central nervous system the disease is characterized by: perivascular infiltration with cells among which lymphocytes and monocytes predominate, focal areas of infiltration, or necrosis and infiltration with neutrophiles. Some of the foci fulfill all the requisites for true suppuration. Whether the cells found in the perivascular infiltrations are recorded as predominately lymphocytes or monocytes may depend upon the organism, the host, and the observer.[1] The foci of neutrophilic infiltration may or may not have vascular relations. Olafson (1940) considers this lesion the most characteristic of the infection, since it is said to contain the bacteria; the perivascular infiltrations do not.

Less constant lesions are focal and perivascular edema or hemorrhage, or both, and degenerative changes in the nerve cells and tracts. Cell inclusions have not been observed; this fact and the fact that attempts to reproduce the disease with bacteria-free filtrates from infected tissues have failed would indicate that a filtrable virus is not involved in listerellosis. That there is some unrecognized factor in natural infection is suggested by the difficulty with which the infection is produced experimentally. The distribution of lesions in the brain suggests the possibility of spread by way of the meninges, canal systems, and vessel sheaths.

Liver lesions when present in cases of listerellosis are noted as discrete foci of necrosis, sometimes preceded by visible fatty changes in the cells (Pirie, 1927) and later surrounded by hepatic cells showing fatty changes (Webb and Barber, 1937). Necrosis is thought to appear in the hepatic cells surrounding Kuppfer cells which have ingested numerous organisms, according to Webb and Barber (1937) and Bianchi (1930). The foci of necrosis enlarge and often become infiltrated with neutrophiles or mononuclear cells, or both. Evidently the necrotic foci usually become infiltrated with leucocytes, among which neutrophiles, large mononuclear cells, or lymphoid cells may predominate.

Myocardial lesions consist of necrosis of the heart muscle and cell infiltrations, among which mononuclears usually predominate.

Eye lesions consist of conjunctivitis, which may be catarrhal or

[1]Many writers use the term "mononuclear cells" in the less restricted sense to include both lymphocytes and monocytes. This use of the term may not be unwise in view of the controversy concerning the differentiation between lymphocytes and monocytes and that concerning the origin of the monocyte. Regarding the origin of the monocyte, Conway (1938) states that: "the free stem cell from which the monocyte develops . . . is morphologically identical with the lymphocytes . . . ," and that: "monoblasts other than these lymphocytes have not been found." Conway's observations are based upon cases of experimental *Listerella* infection in rabbits and guinea pigs.

follicular in nature; keratitis, characterized principally by destruction of epithelium and mild infiltration with leucocytes, mainly neutrophiles; and extension of the capillary system into the substantia propria of the cornea.

Other lesions have seldom been observed or have not been well described.

STUDIES ON IMMUNIZATION

After listerellosis was recognized to be a fairly common disease of ruminants, research on immunization was initiated. Bacterins used under uncontrolled conditions in field outbreaks of listerellosis among Illinois sheep and cattle gave doubtful results, as has been pointed out in the description of these outbreaks. Olafson (1940) reported that in New York bacterins had been used in some affected flocks, but that the disease usually disappeared before the bacterins showed any value. In twelve to eighteen treatments over a period of 6 weeks Olafson injected 20 sheep with alternate subcutaneous and intravenous doses of 2.5 cc. of presumably living broth cultures of *Listerella* (no mention is made that the cultures were killed). Ten days after the last subcutaneous or intravenous inoculation the sheep were exposed by the intracerebral route. After intracerebral exposure, 6 sheep soon died, 4 became very ill but recovered, 4 become ill 23 to 24 days later and died, and 6 remained healthy.

Living and Killed Listerella Cultures[1]

Rabbits and guinea pigs. *Listerella* Strain 27681 (Paterson Type 1), isolated from an aborted bovine fetus, and Strain 26307 (Paterson Type 4), isolated from the brain of a sheep, were employed in making a bivalent bacterin for vaccinating rabbits and guinea pigs. The bacteria were grown for 48 hours at 37° C. in beef infusion broth containing 5-percent horse serum, and they were then killed by adding .5-percent formalin. The density of the resultant bacterin was adjusted to that of the McFarland Nephelometer Tube 1.

Twelve rabbits in three groups of 4 each were used in the first experiment. All the rabbits were first inoculated subcutaneously with the bivalent bacterin at weekly intervals. The first group was inoculated twice with doses of 1 cc. each; the second group three times with doses of 2 cc. each; and the third group four times with doses of 3 cc. each.

After the subcutaneous inoculations the rabbits were exposed to living *Listerella* by intravenous injection with .1 cc. of a saline suspension of *Listerella* Strain 27681. The rabbits in the first group were

[1]Some of these observations have already been reported by Graham, Morrill, and Levine (1940) and by Graham and Levine (1942).

exposed 23 days after the last subcutaneous inoculation, those in the second group 16 days after, and those in the third group 9 days after. The strain of *Listerella* used for exposure had previously been titrated by intravenous inoculation of normal rabbits in order to determine the dosage which would consistently kill. Dosages of .01 cc. to .4 cc. of the saline suspension had been tested, and it had been ascertained that .01 cc. failed to kill but dosages of .1 cc. to .4 cc. were always fatal.

The 4 rabbits in the first group died in 2 to 7 days after exposure, those in the second group died or were destroyed in a moribund state in 3 to 9 days, and those in the third group in 2 to 9 days. Subcutaneous vaccination failed to protect rabbits against subsequent intravenous exposure to *Listerella.*

Sixteen rabbits in four groups of 4 each were employed in the second experiment. At weekly intervals the first three groups were inoculated subcutaneously with 1-cc. doses of the bivalent bacterin; the fourth group was left untreated as a check. The first group was given two doses, the second group three doses, and the third group four doses. Thirty-two days after the first subcutaneous injection was given, all 16 rabbits were exposed to *Listerella* Strain 27681 by the conjunctival route. One drop of a saline suspension (density McFarland Nephelometer Tube 3) was placed in one eye of each rabbit. Of the 4 rabbits in the first group, 3 exhibited conjunctivitis (mild in 2) and 1 exhibited conjunctivitis and keratitis. Of the 4 rabbits in the second group, 3 exhibited conjunctivitis and 1 conjunctivitis and keratitis. Of the 4 rabbits in the third group, 2 exhibited conjunctivitis, 1 conjunctivitis and keratitis, and 1 failed to react. Of the 4 rabbits in the fourth or control group, 1 exhibited conjunctivitis and 3 conjunctivitis and keratitis. In some of the rabbits, conjunctivitis also appeared in the untreated eye, indicating contact infection.

Sixty-three days after the first exposure, a second supraconjunctival exposure, this time of both eyes, was carried out on 10 of the 16 rabbits (3 from the first group, 3 from the second, 2 from the third, and 2 from the fourth group). Of the 10 rabbits exposed the second time, 2 remained free of ocular symptoms, 4 exhibited conjunctivitis, and 4 showed both conjunctivitis and keratitis. No significant difference was noted between the reactions in the right and left eyes, indicating that any immunity which may have been induced by the previous exposure had disappeared.

The second experiment was repeated with 16 more rabbits, but two modifications were made. Preliminary subcutaneous injections were timed so that they were all completed on the same day. All groups were then divided in half, one half being exposed 23 days and the other half 31 days after the last inoculation. After exposure 2 rabbits, 1 of which was from the control group, failed to exhibit ocular symptoms; 10 rabbits exhibited conjunctivitis only; and 4, both conjunctivitis

and keratitis. Four rabbits that developed conjunctivitis showed reaction in the eye which had not been exposed artificially. No significant difference was noted between the rabbits exposed 23 days after the last vaccination and those exposed 31 days after. As judged by promptness and severity of the ocular reaction, the control animals were, if anything, less susceptible than the vaccinated ones. The results of the two experiments indicate that repeated subcutaneous inoculation of rabbits with *Listerella* bacterin prepared as indicated has no effect on the results of subsequent supraconjunctival exposure.

A similar experiment was set up with guinea pigs. Sixteen animals were divided into four groups of 4 each. The guinea pigs in the first group were given two subcutaneous doses of 1 cc. each of the bivalent bacterin a week apart; the fourth group was used as untreated controls. The 8 guinea pigs in the second and third groups were given three subcutaneous injections and then they died before they could be exposed. Thirty days after the last subcutaneous inoculation the guinea pigs in the first and fourth groups were exposed supraconjunctivally with one drop of a saline suspension of *Listerella* Strain 27681 (density McFarland Nephelometer Tube 3). All animals developed conjunctivitis and keratitis following exposure, indicating that the single subcutaneous injection of bacterin had no protective action against subsequent supraconjunctival exposure.

Chickens. To test their efficacy against intracerebral exposure, living and killed *Listerella* suspensions were administered subcutaneously to 125 chickens ranging in age from 5 to 6 weeks. The chickens were divided into five groups of 25 each. The birds in the first three groups were inoculated subcutaneously with 1-cc. doses of a formalin-killed saline suspension of *Listerella* Strain 10957 (density McFarland Nephelometer Tube 10). The first group was inoculated once; the second group twice at a 5-day interval; the third group three times at the same intervals. The chickens of the fourth group were given one 1-cc. dose each of saline suspension of living *Listerella.* The fifth group was left untreated as a check.

The *Listerella* culture used in exposing these birds was grown in beef broth for 20 hours, dehydrated *in vacuo,* and stored at —1° C. The dosage was determined by intracerebral inoculation of a series of susceptible chickens. Before inoculation the dehydrated culture was diluted with physiological salt solution to densities from 1-100 to 1-10,000 that of a suspension having a Gates gage reading of 10.2. The exposure dose in each case was .1 cc. Three chickens died following intracerebral injection with the 1-100 dilution, 2 of 3 with the 1-1,000 dilution, and 1 of 3 chicks with the 1-10,000 dilution.

Six weeks after the first subcutaneous inoculation was given, all 125 chickens were inoculated intracerebrally with .1 cc. of a saline suspension of *Listerella* (density 1-10 Gates 10.2). Almost all the birds were

dead 3 days later, and the remainder died during the next few days. Thus, neither repeated subcutaneous administration of bacterin nor a single subcutaneous inoculation with living *Listerella* protected chickens against subsequent intracerebral exposure.

Sheep. The value of subcutaneous inoculation with a *Listerella* bacterin against subsequent intravenous exposure was studied in an experiment in which 8 lambs and 5 ewes were employed. A bivalent bacterin was prepared from ovine *Listerella* Strain 10957 and bovine Strain 12159 by growing the bacteria for 48 hours at 37° C. in beef infusion broth containing horse serum. The culture was killed by the addition of .5-percent formalin and then it was adjusted to the density of the McFarland Nephelometer Tube 1. Five lambs were given two subcutaneous doses of 3 cc. each at a 4-day interval. Three ewes were given two subcutaneous doses of 5 cc. each at the same interval. Three lambs and 2 ewes were not vaccinated.

Two weeks after the first inoculation all ewes and lambs were exposed intravenously with 1 to 4 cc. (depending upon their weight) of a saline suspension of *Listerella* Strain 27681 (density McFarland Nephelometer Tube 5). At the time of intravenous exposure a drop of the culture was also placed in one eye of each animal. All the sheep were sick and stiff 2 days after exposure. Their temperatures were elevated and they refused food. The 8 vaccinated animals died 6 to 28 days after exposure (Table 20); and 4 of the 5 unvaccinated sheep

TABLE 20.—RESULTS OF SUBCUTANEOUS INOCULATION OF SHEEP WITH LISTERELLA BACTERIN AGAINST SUBSEQUENT INTRAVENOUS EXPOSURE

(Bacterin: formalin-killed saline suspension with a density of McFarland Nephelometer Tube 1; exposure: suspension of living *Listerella* with a density of McFarland Nephelometer Tube 5)

Animal No.	Weight of animal	Bacterin		Exposure dose	Days from exposure to death
		Number of doses	Size of dose		
	lb.		*cc.*	*cc.*	
Lamb 21	26	2	3	1	6
Lamb 26	26	2	3	1	6
Lamb 38	21	2	3	1	6
Lamb 41	20	2	3	1	6
Lamb 42	21	2	3	1	6
Ewe 1920	95	2	5	3	13
Ewe 1928	139	2	5	4	28
Ewe 1977	73	2	5	3	7
Ewe 1705	115	..	..	4	4
Ewe 1519	120	..	..	4	8
Lamb 1934	28	..	..	1	7
Lamb 1938	25	..	..	1	6
Lamb (wether)	90	..	..	3	(Survived; slight illness only)

died 4 to 8 days after exposure. One unvaccinated wether became ill but recovered. The results indicate that vaccination had no effect upon the resistance of the sheep to subsequent intravenous exposure to *Listerella.*

The blood of these animals was studied during the course of the experiment. After exposure the total leucocyte count decreased slightly in 3 lambs and 1 ewe; it decreased markedly in 1 ewe; it increased at first in 3 lambs and 2 ewes and then dropped, returning to the original level in 1 lamb and passing below that level in the other animals. No significant change in total leucocyte counts was observed in 1 lamb, 1 ewe, and the wether that recovered.

Differential blood counts indicated an increase in percentage of neutrophiles in 5 lambs, 3 ewes, and the wether. No significant change in the differential counts was observed in the other 2 lambs and 2 ewes. In no case did the percentage of monocytes increase significantly. There was no significant difference between the vaccinated and unvaccinated animals in total or differential leucocyte counts.

This vaccination experiment with sheep suggests that the apparently favorable results obtained in the uncontrolled field vaccination may have been coincidental, the small doses of vaccine employed being without real value. Hence more conclusive information on the prophylactic efficacy of *Listerella* bacterin was sought by administering large doses of it to sheep in a flock in which listerellosis had occurred in two previous years (for a detailed history of the flock, see page 10). Briefly, listerellosis had caused mortalities of approximately 5 to 6 percent in this flock during the winters of 1938-39 and 1940-41.

At the time of experimental vaccination in September, 1941, the flock consisted of approximately 1,000 ewes. A bacterin was made from a *Listerella* strain isolated in 1941 from a typical case of *Listerella* encephalitis in this herd. It was grown for 48 hours at room temperature in beef broth and killed with .5-percent formalin. Its density was equivalent to that of the McFarland Nephelometer Tube 3.

The ewes were divided into four lots. The first three lots were inoculated subcutaneously with 20-cc. doses of the bacterin. Where more than one dose was given, injections were made at weekly intervals. Lot 1 (175 yearlings and 101 grade Shropshires) received a single dose; Lot 2 (175 yearlings and 90 grade Hampshires and Southdowns), two doses; and Lot 3 (55 yearlings, 150 crossbreds and Merinos, and 116 westerns), three doses. The ewes in Lot 4 (91 yearlings and 71 crossbred Merinos) were not treated and served as controls.

No artificial exposure was carried out. After treatment, the sheep were kept in presumably contaminated lots and sheds where the disease had occurred before. They remained there all winter and during the first part of the spring.

TABLE 21.—EFFECTS OF SUBCUTANEOUS INOCULATION OF EWES WITH LISTERELLA BACTERIN ON SUBSEQUENT INCIDENCE OF THE DISEASE

Lot No.	Number of ewes	Dosage of bacterin	Deaths from listerellosis	
			Number	Percent
		cc.		
1	276	20	12	4.4
2	265	40	13	4.9
3	322	60	11	3.4
4	162	0	10	6.2

Listerellosis (confirmed by isolation of the organism from the brains of typically affected animals) occurred among the sheep in all four lots (Table 21). It first appeared in January, 1942, and losses continued until the sheep were placed on pasture in April. Deaths from the disease amounted to 4.4 percent in Lot 1, 4.9 percent in Lot 2, 3.4 percent in Lot 3, and 6.2 percent in the unvaccinated Lot 4.

A statistical analysis[1] showed that there was no significant difference between the death losses in Lots 1 and 2 and the control Lot 4. The difference in losses between Lot 3, in which the sheep received a total of 60 cc. of bacterin, and the control Lot 4 was probably significant, since the analysis indicated that it would occur only once in 25 times if chance variation alone were responsible. However, the immunogenic action of the bacterin was relatively feeble, and vaccination against listerellosis, even with the large doses of bacterin employed in this experiment cannot be recommended as practical.

Experimental Antiserum

The use of antiserum against *Listerella* has received relatively little attention. Graham, Morrill, and Levine (1940) reported one experiment on attempted passive immunization of rabbits and guinea pigs. Julianelle (1941A) was unable to protect mice against *Listerella* infection by mixing the organisms with antiserum (titer 1-5,120) prior to intraperitoneal inoculation.

Rabbits and guinea pigs. In the first experiment with antiserum, 10 guinea pigs and 10 rabbits were used. Four rabbits and 4 guinea pigs were inoculated subcutaneously with doses ranging from .5 cc. to 3 cc. of antiserum (titer 1-50,000) from a cow which had received repeated injections of *Listerella* Strain 27681. Two days later, all 8 animals were exposed by subcutaneous inoculation with .1 cc. of a saline suspension of living *Listerella* (density McFarland Nephelom-

[1]The statistical analysis was made by H. W. Bean of the Department of Animal Husbandry, College of Agriculture, University of Illinois, using the chi-square method.

eter Tube 1). On the day of exposure 4 more rabbits and 4 more guinea pigs that had not been treated previously were inoculated subcutaneously with mixtures of .1 cc. of the living *Listerella* suspension and .5 cc. to 3 cc. of the antiserum which had been incubated together at 37° C. for 30 minutes before administration. Two control rabbits and 2 control guinea pigs were also given .1 cc. of the living *Listerella* suspension subcutaneously.

One control guinea pig died 16 days after exposure, but *Listerella* was not isolated from it on culture. All the other animals remained healthy. Three weeks after exposure, the 19 remaining rabbits and guinea pigs were each inoculated subcutaneously with .5 cc. of a living *Listerella* suspension (density McFarland Nephelometer Tube 10). Sixteen of the animals survived for 22 days, at which time 15 of them and 4 fresh controls were inoculated with .8 cc. of a living *Listerella* suspension (density McFarland Nephelometer Tube 10). One control and 5 of the previously exposed animals survived.

Since the above experiment indicated that too small an initial exposing dose had been used, the experiment was repeated with other animals according to the same plan except that an exposing dose of .2 cc. of a living *Listerella* (density McFarland Nephelometer Tube 10) was employed. Six of the 8 animals which had received antiserum died, and *Listerella* was isolated from 5 of them on culture. All the control animals remained healthy. Three weeks after exposure the 14 survivors and 4 new controls were given subcutaneously .5 cc. of a living *Listerella* suspension (density McFarland Nephelometer Tube 10). All the control guinea pigs died and all the previously inoculated guinea pigs except one, but all the rabbits remained healthy.

In a third experiment a bovine antiserum (titer 1-6,400), prepared against *Listerella* Strain 27681 was employed in the attempted immunization of rabbits. Two rabbits received subcutaneously two 4-cc. doses each of the antiserum 2 days apart. The next day these animals together with 2 controls were exposed by subcutaneous inoculation with 2 cc. each of a saline suspension of *Listerella* Strain 27681 (density McFarland Nephelometer Tube 10). The control rabbits survived, but 1 rabbit which had received 4 cc. of antiserum died 7 days after exposure, and another rabbit which had received 8 cc. of antiserum died 10 days after exposure.

In a fourth experiment a bovine antiserum (titer 1-8,000), prepared against *Listerella* Strain 27681, was employed subcutaneously. Two rabbits received a single 2-cc. dose of the antiserum; 2 received two 2-cc. doses 3 days apart; and 2 others received three 2-cc. doses at intervals of 4 and 3 days. Four days after the last injection all the treated rabbits, together with the 6 controls, were exposed by subcutaneous inoculation with 2 or 3 cc. of a saline suspension of living *Listerella* Strain 27681 (density 1.5 to 2 times that of the McFarland

Nephelometer Tube 10). No significant difference was observed between the results with the larger and smaller doses. The 2 rabbits which had received 2 cc. of the antiserum died, one 4 days and the other 12 days after exposure. The 2 rabbits which had received 4 cc. of antiserum died, one 8 days and the other 15 days after exposure. One rabbit which had received 6 cc. of antiserum died 12 days after exposure but the other survived. Two of the control rabbits died, one 6 days and the other 7 days after exposure, but the other 4 control rabbits survived.

Thus in the third and fourth experiments 7 out of 10 rabbits treated with antiserum died following subcutaneous exposure to *Listerella,* but only 2 out of 8 control rabbits died following exposure. It was thought that perhaps the heterologous serum might have influenced the result. Accordingly a comparison of the actions of bovine and rabbit antisera was made.

In a fifth experiment made to compare bovine and rabbit antisera, 3 rabbits were inoculated subcutaneously with 2 cc. of bovine antiserum (titer 1-12,800) prepared against *Listerella* Strain 27681. Three rabbits received two similar doses 4 days apart. Another lot of 3 rabbits was inoculated subcutaneously with 2 cc. of a rabbit antiserum (titer 1-2,000) prepared against Strain 27681, and 3 additional rabbits were inoculated with two similar doses 4 days apart.

Three days after the last inoculation 1 rabbit from each of the four groups described and 3 rabbits not inoculated were exposed by subcutaneous inoculation with 2 cc. of a saline suspension of living *Listerella* Strain 27681, which contained .6 percent of organisms by volume. The rabbit which had received 2 cc. of bovine antiserum died 37 days after exposure; the rabbit which had received 4 cc. of the same kind of antiserum died 4 days after exposure; but the 2 rabbits which had received rabbit antiserum and the 3 control rabbits survived.

Eleven days after the second series of inoculations, the other 2 rabbits in each of the four groups and 3 controls not inoculated previously were subcutaneously exposed to 2.4 cc. of a saline suspension of living *Listerella* Strain 27681 which contained .5 percent organisms by volume. Of the rabbits inoculated in the third series, 1 of the 2 which had received 2 cc. of bovine antiserum died 7 days after exposure and the other survived. The 2 rabbits which had previously received 4 cc. of bovine antiserum died, one 6 days and the other 7 days after exposure; the 4 rabbits which had received rabbit antiserum all survived. One of the control rabbits died 22 days after exposure; the other 2 survived.

Thus only 1 out of 6 rabbits which had received bovine antiserum survived exposure to *Listerella,* but all 6 rabbits which had received rabbit antiserum survived exposure, as did 5 out of 6 controls. The smaller survival after treatment with the bovine antiserum could not

be due to a low agglutinin concentration in the basic antiserum, since its titer (1-12,800) was considerably higher than that of the rabbit antiserum (1-2,000).

Altho Julianelle (1940) states that there is no relationship between the local ocular reaction to *Listerella* and the antibody titer of the circulating blood, it was felt worth-while to study the effect of antiserum upon the results of conjunctival exposure.

In a preliminary experiment, a bovine antiserum (titer 1-50,000) prepared against *Listerella* Strain 27681 was used subcutaneously. One rabbit and 1 guinea pig were each given 1 cc. of the antiserum; 1 rabbit and 1 guinea pig received 2-cc. doses; and 1 rabbit and 1 guinea pig received 3-cc. doses. Two days after this treatment, all animals, together with a control rabbit and a control guinea pig, were exposed by instillation into the conjunctival sac of one drop of a suspension of living *Listerella* Strain 27681 (density McFarland Nephelometer Tube 3). In both inoculated and control animals conjunctivitis and, in some individuals, keratitis developed.

The experiment was repeated with 4 more rabbits and 4 more guinea pigs. A bovine antiserum with a titer of 1-6,400 was used and the animals were exposed 3 days after injection of antiserum. Conjunctivitis and keratitis appeared in the control rabbit and in the rabbit which had received 1 cc. of antiserum. The rabbit which had received 2 cc. of antiserum developed keratitis and died 19 days after exposure. *Listerella* was isolated from its brain. The rabbit which had received 3 cc. of antiserum exhibited only conjunctivitis. The control guinea pig and the guinea pig which had received 3 cc. of antiserum developed conjunctivitis and keratitis, and the cornea ruptured in both animals. The guinea pigs which had received 1 cc. and 2 cc. of antiserum developed conjunctivitis and keratitis, the one given the 1-cc. dose dying 15 days after exposure and the other 11 days after exposure. *Listerella* was recovered from the heart blood of the second animal.

Thus in the experiment just described death resulted in 1 of 3 antiserum-treated rabbits and in 2 of 3 antiserum-treated guinea pigs following supraconjunctival exposure; the control animals survived. While these results suggest that the antiserum produced sensitization, too few animals were used to draw this conclusion. This experiment, together with the previous one, however, furnished no evidence of protective action by the antiserum.

Since bovine antiserum failed to protect rabbits and guinea pigs against supraconjunctival exposure to *Listerella,* antisera prepared from 2 horses were employed in the next experiments.

Before injections with *Listerella* were instituted, serum was obtained from the two horses for a check test. Two rabbits were inoculated subcutaneously with serum from Horse SB. One was given a

2-cc. dose and the other a 4-cc. dose. One week after treatment these two animals and a third control rabbit were exposed to *Listerella* Strain 39347 (Paterson Type 4) by instilling into each eye .05 cc. of a saline suspension (density McFarland Nephelometer Tube 5). In addition, a portion of the saline suspension was mixed with an equal

TABLE 22.—RESULTS OF SUBCUTANEOUS INOCULATION OF RABBITS WITH EQUINE LISTERELLA ANTISERUM AGAINST SUBSEQUENT SUPRACONJUNCTIVAL EXPOSURE

(Exposure one week after serum treatment; a saline suspension of *Listerella* Strain 39347 with a density of McFarland Nephelometer Tube 5 was used)

Rabbit No.	Total dosage of *Listerella* culture given the horse before serum was collected		Equine antiserum used	Exposure dose	Results[a]
	Formalin-killed	Living			
Antiserum from Horse SB					
	cc.	*cc.*	*cc.*	*cc.*	
1	0	0	2	.1	−
2	0	0	4	.1	+ left eye
3	.....	....	0	.1[b]	2+ both eyes
4	.....	....	0	.1	3+ both eyes
5	70.0	7.5	2	.1	4+ both eyes
6	70.0	7.5	4	.1	4+ both eyes; death in 11 days
7	.....	....	0	.1[b]	−
8	.....	....	0	.1	4+ both eyes
9	70.0	39.5	2	.1	4+ both eyes
10	70.0	39.5	4	.1	4+ both eyes
11	.....	....	0	.1[b]	−
12	.....	....	0	.1	−
Antiserum from Horse C					
1	0	0	2	.1	3+ right eye
2	0	0	4	.1	3+ both eyes
3	.....	....	0	.1[b]	−
4	.....	....	0	.1	3+ both eyes
5	140.0	15.0	2	.1	4+ both eyes
6	140.0	15.0	4	.1	2+ both eyes
7	.....	....	0	.1[b]	−
8	.....	....	0	.1	4+ both eyes
9	140.0	79.0	2	.1	+ both eyes
10	140.0	79.0	4	.1	+ both eyes
11	.....	....	0	.1[b]	−
12	.....	....	0	.1	+ both eyes
13	177.5	79.0	2	.05[c]	3+ left eye
14	177.5	79.0	2	.05[c]	3+ left eye
15	177.5	79.0	4	.05[c]	3+ left eye
16	177.5	79.0	4	.05[c]	3+ left eye
17	.....	....	0	.05[c]	+ left eye
18	.....	....	0	.05[c]	−
19	.....	....	0	.05[c]	−
20	.....	....	0	.05[c]	−

[a]A − sign indicates no reaction, + slight conjunctivitis, 2+ slight conjunctivitis with serous discharge, 3+ purulent conjunctivitis, 4+ purulent conjunctivitis with profuse discharge. [b]A *Listerella* culture that had been mixed with an equal volume of serum and incubated 30 minutes at 37° C. was used instead of the saline suspension. [c]Only the conjunctiva of the left eye was exposed.

amount of serum, incubated for 30 minutes at 37° C., and .1 cc. of the mixture was instilled into the eye of a fourth rabbit. Four more rabbits were employed similarly in a test of the serum of Horse C.

The rabbit which had received 2 cc. of serum from Horse SB failed to develop conjunctivitis, but the one that had received 4 cc. of the same serum had slight conjunctivitis of one eye. The rabbit given the serum-culture mixture exhibited slight bilateral conjunctivitis with a mucous discharge. A bilateral purulent conjunctivitis appeared in the control rabbit (Table 22).

The rabbits which had received serum from Horse C and the control rabbit developed bilateral purulent conjunctivitis with an abundant discharge. The one that had received 4 cc. of serum died 11 days after exposure. The one that had been exposed with the serum-culture mixture failed to show symptoms.

Horse SB was inoculated subcutaneously at weekly intervals with 10 cc., 20 cc., and 40 cc. of a formalin-killed culture of *Listerella* Strain 39347 followed at weekly intervals with .5 cc., 1 cc., 2 cc., and 4 cc. of a saline suspension of living *Listerella* (density McFarland Nephelometer Tube 5). Horse C received similar inoculations with doses of 20 cc., 40 cc., and 80 cc. of the bacterin and 1 cc., 2 cc., 4 cc., and 8 cc. of the living culture. Two weeks later serum was obtained from both horses for protection tests, which were set up in the same way as the tests for the normal horse sera (4 rabbits for each test).

The rabbits which had received antiserum from Horse SB and the control rabbit developed a bilateral purulent conjunctivitis with abundant discharge from the eyes following exposure. The rabbit that had received 4 cc. of antiserum died 11 days after exposure. The rabbit that was exposed with the antiserum-culture mixture showed no symptoms.

A bilateral purulent conjunctivitis with a profuse discharge from the eyes developed in the rabbit that had received 2 cc. of antiserum from Horse C and the control rabbit. No symptoms were observed in the rabbit that was exposed with the antiserum-culture mixture.

Eighteen days after the last treatment described above, both horses were subjected to a further series of inoculations. Horse SB received eight subcutaneous doses of 4 cc. each of the living *Listerella* suspension at semiweekly intervals; Horse C received eight doses of 8 cc. each, also given subcutaneously at semiweekly intervals. Serum was obtained from both horses 11 days after the last injection and rabbit protection tests were made in the same way as the earlier tests.

The 2 rabbits which had received subcutaneous injections of antiserum from Horse SB developed bilateral purulent conjunctivitis with a profuse discharge from the eyes following exposure. The rabbit which had been exposed with the antiserum-culture mixture and the control rabbit exhibited no symptoms.

The rabbits which had received subcutaneous injections of antiserum from Horse C and the control rabbit developed slight bilateral conjunctivitis following exposure; no symptoms appeared in the rabbit which had been exposed with the antiserum-culture mixture.

Approximately five months after the last injection described above, Horse C was subjected to a further series of inoculations. He was given subcutaneous doses of .5 cc., 2 cc., 5 cc., and 10 cc. of formalin-killed *Listerella* bacterin at semiweekly intervals. The day after the last inoculation, serum was obtained for a rabbit protection test.

In this test of the serum from Horse C, 2 rabbits received 2 cc. each of the antiserum subcutaneously; 2 rabbits received 4 cc. each also subcutaneously; and 4 rabbits served as controls. All 8 animals were exposed to *Listerella* by supraconjunctival inoculation of the left eye one week after treatment, as previously described. The 4 rabbits that had received antiserum developed purulent conjunctivitis following exposure. One of the control rabbits developed a slight conjunctivitis; the other three controls remained normal.

The results obtained with equine *Listerella* antiserum were quite similar to those obtained with bovine antiserum. Subcutaneous inoculation with the antiserum failed to protect rabbits against subsequent conjunctival exposure. Indeed there is a suggestion that it might even have sensitized them to the disease. None of the rabbits exposed with the antiserum-culture mixture developed conjunctivitis. Altho the number of rabbits treated with the mixture was small, this observation suggests that the antiserum may possess some neutralizing power *in vitro*.

Sheep and cattle. A limited experiment was carried out on the value of antiserum in protecting calves and lambs against intravenous exposure to *Listerella*. A bovine antiserum (titer 1-12,800) was employed. One calf received two 25-cc. doses of the antiserum subcutaneously a week apart. One lamb received a single 20-cc. dose, and another lamb received two 20-cc. doses a week apart. One week after the second injection with antiserum these animals, together with 1 untreated calf and 2 untreated lambs, were exposed to *Listerella*. The calves received 2 cc. each and the lambs 1 cc. each of an intravenous dose of a saline suspension of living *Listerella* (density .5 percent organisms by volume for the calves and .6 percent organisms by volume for the lambs).

Febrile reactions were observed in both the treated and control animals (Table 23). The calf which had received antiserum died 5 days after exposure; the lamb which had received 20 cc. of antiserum died 21 days after exposure; the lamb which had received 40 cc. of antiserum was destroyed when moribund 55 days after exposure; and 1 control lamb died 34 days after exposure. *Listerella* was recovered on culture from all animals which died or became moribund. The con-

TABLE 23.—RESULTS OF INOCULATING CALVES AND LAMBS WITH BOVINE LISTERELLA ANTISERUM AGAINST SUBSEQUENT INTRAVENOUS EXPOSURE

(Preliminary inoculation: a bovine antiserum with a titer of 1-12,800; exposure: a saline suspension of living *Listerella* of .5 percent organisms by volume was used for the calves and .6 percent for the lambs)

Animal No.	Amount of antiserum used	Exposing dose	Results
	cc.	*cc.*	
Calf 1	25 and 25	2	Febrile reaction; death after 5 days[a]
Calf 2	0	2	Febrile reaction; recovered
Lamb 1	20	1	Febrile reaction; death after 21 days[a]
Lamb 2	20 and 20	1	Febrile reaction; destroyed when moribund after 55 days[a]
Lamb 3	0	1	Febrile reaction; death after 34 days[a]
Lamb 4	0	1	Febrile reaction; recovered

[a] *Listerella* was recovered on culture.

trol calf and the second control lamb recovered and appeared healthy when finally released.

The above results indicate that the antiserum had no prophylactic value against intravenous exposure of calves and lambs, and even suggested, as in the experiment with rabbits, that it might have sensitized the animals.

SUMMARY

This monograph is an attempt to bring together all available information on the diverse manifestations of disease associated with *Listerella* infection in different hosts. The reports of other workers, as well as data from the Illinois Station, have been presented.

Listerellosis incurred under natural conditions is a systemic disease in rodents and chickens. In man it is characterized chiefly by meningitis, in ruminants and swine by encephalitis. Abortion in cattle and sheep has also been reported as due to *Listerella,* and these reports have been supported by experimental findings.

In its usual encephalitic form, listerellosis can be diagnosed quite readily by bacteriologic examination of the medulla at autopsy. Once the diagnosis has been established in a herd, the clinical symptoms make it possible to recognize affected animals quite easily.

Listerellosis occurs most commonly in winter and early spring when the animals are closely confined. No universally effective preventive measures are known. Isolation of the sick animals seems effective in some herds; in others, the disease fails to disappear until the animals are turned out on pasture. Thus it is quite evident that the epizoology of listerellosis deserves further investigation, including particularly the natural reservoir of infection and mode or modes of transmission.

According to reports from veterinary clinicians, the sulfonamide drugs may be of value in treating cattle for listerellosis but only if they are administered very early and in large doses. Once encephalitic symptoms appear it is probably too late to institute therapy with much hope of success. The problem then becomes one of recognizing new cases and starting treatment as early as possible. The most promising approach to this problem is probably a study of the body temperatures of apparently normal animals in affected herds, since the temperature usually is elevated early in the disease.

Since abortion associated with *Listerella* infection has been recognized in cattle and sheep, cases of abortion in ruminants should be investigated to see if the organism is present, particularly if there is a herd history of encephalitis.

Results of studies on listerellosis made at the Illinois Station may be summarized as follows:

1. The disease manifested itself as an encephalitis in sheep in the seven outbreaks reported in Illinois and as an encephalitis also in cattle in seven of the eight outbreaks reported in the state. In the exceptional outbreak in cattle the infection was associated with abortion. The disease in the one outbreak reported in chickens was systemic.

2. The causative organism, *Listerella monocytogenes,* is Gram-positive and rod-shaped. It is approximately .5 by 1 micron in size, exhibits a rather peculiar tumbling motility, and causes beta hemolysis. Growth occurs quite well at room temperature as well as at 37° C.

3. Serologic studies indicated that neither the agglutination nor the complement fixation test was of value in the diagnosis of listerellosis in cattle. Serologic studies in horses failed to reveal any relation between *Listerella* and equine recurrent (periodic) ophthalmia.

4. In experimental listerellosis in cattle, sheep, rabbits, guinea pigs, swine, horses, and chickens, the distribution of lesions varies with the route of inoculation. Conjunctivitis and keratitis, which are occasionally observed in the natural disease in ruminants, may be quite readily induced in rabbits and guinea pigs by supraconjunctival inoculation. This characteristic of *Listerella* is an aid in identifying the organism.

5. Histopathologic studies indicate that the essential character of the lesion in a given tissue is the same in the experimental infection as in the naturally incurred disease. In the brain, lesions are likely to be more numerous in the white than in the gray matter and are constituted mainly by focal infiltrations of neutrophiles and by perivascular infiltration of lymphoid and mononuclear cells. In animals which develop a systemic infection, foci of necrosis and infiltration with lymphoid and mononuclear cells may be observed in the liver and heart.

6. Attempts at immunization of rabbits, guinea pigs, chickens,

and sheep against listerellosis by means of killed (and in some cases living) *Listerella* cultures were unsuccessful.

7. Attempts at immunization of rabbits, guinea pigs, sheep, and cattle against listerellosis by means of antisera were unsuccessful. In some cases the administration of antiserum apparently rendered the animals even more susceptible to infection.

8. Little success was obtained in the treatment of clinically affected sheep with sulfanilamide.

BIBLIOGRAPHY

Anton, W., (1934) Kritisch-experimenteller Beitrag zur Biologie des *Bakterium monocytogenes*. Mit besonderer Berücksichtigung seiner Beziehung zur infektiösen Mononukleose des Menschen. Zentbl. f. Bakt. [etc.] Abt. I, Orig. **131**, 89-103.

Atkinson, E. (1917) Meningitis associated with Gram-positive bacilli of diphtheroid type. Med. Jour. Austral. **1**, 115-118 [cited by Julianelle, 1940].

Baldridge, C. W., Rohner, F. J., and Hansman, G. H. (1926) Glandular fever (infectious mononucleosis). Arch. Int. Med. **38**, 413-448.

Barber, Mary (1939) A comparative study of *Listerella* and *Erysipelothrix*. Jour. Path. and Bact. **48**, 11-23.

Becker, E. R. (1939) Duplication of generic names among Protista. Rpt. to Com. on Nomenclature at 3d Internatl. Cong. for Microbiol., New York, 1939.

Bianchi, L. (1930) Ricerche sperimentali ed isto-patologiche sull'infezione da *Bacterium monocytogenes* nel coniglio. Haematol. **11**, 163-188.

Biester, H. E., and Schwarte, L. H. (1939) Studies on *Listerella* infection in sheep. Jour. Infect. Dis. **64**, 135-144.

——— ——— (1940) *Listerella* infection in swine. Amer. Vet. Med. Assoc. Jour. **96**, 339-342.

——— ——— (1941A) Listerellosis in swine and criteria for diagnosis. U. S. Livestock Sanit. Assoc. Rpt. 1940, pp. 42-47.

——— ——— (1941B) Bovine listerellosis in Iowa with studies on a recovered case. North Amer. Vet. **22**, 729-734.

Bloom, W. (1928) The origin and nature of the monocyte. Folia Haematol. **37**, 1-62.

Burn, C. G. (1934) Unidentified Gram-positive bacillus associated with meningoencephalitis. Soc. Exp. Biol. and Med. Proc. **31**, 1095.

——— (1935) Characteristics of a new species of the genus *Listerella* obtained from human sources. Jour. Bact. **30**, 573-591.

——— (1936) Clinical and pathological features of an infection caused by a new pathogen of the genus *Listerella*. Amer. Jour. Path. **12**, 341-348.

Carey, B. W., Jr. (1936) Infection with an organism of the genus *Listerella*. Report of a case of acute cerebrospinal meningitis with recovery. Jour. Ped. **8**, 626-629.

Cislaghi, F. (1938) Su di un caso di meningite acuta linfocitaria benigna con mononucleosi ematica. Pediatria **46**, 637-641 [cited by Julianelle, 1940].

Cole, R. K. (1941) Listeria (*Listerella*) infection in the fowl. Poultry Sci. **20**, 28-31.

Conway, E. A. (1938) Reaction of lymphatic tissues in early stages of *Bacterium monocytogenes* infection. Arch. Path. **25**, 200-227.

——— (1939) Reaction of lymphatic tissue of rabbits to repeated injections of *Bacterium monocytogenes*. Jour. Infect. Dis. **64**, 217-240.

Cromwell, H. W., Sweebe, E. E., and Camp, T. C. (1939) Bacteria of the *Listerella* group isolated from foxes. Science **89**, 293.

Cross, Floyd (1941) New sheep disease diagnosed by station. Colo. Farm. Bul. **3**, No. 3, p. 9.

Doyle, L. P. (1932) Encephalitis in sheep. Amer. Vet. Med. Assoc. Jour. **81**, 118.

Dumont, J., and Cotoni, L. (1921) Bacille semblable au bacille du rouget du porc recontré dans le liquide céphalo-rachidien d'un méningitique. Inst. Past. [Paris] Ann. **35**, 625-633.

EVANS, W. M., and SAWYER, J. E. (1942) Bovine abortion apparently due to *Listeria* infection. Cornell Vet. **32**, 448-449.

FINCHER, M. C. (1935) Meningo-encephalitis of ruminants. Cornell Vet. **25**, 61-63.

FISCHER, J. T. (1941) Las meningoencefalitis a *Listerella monocitogens;* a proposito del primer caso identificado en Sud América. Arch. Uruguayas de Med. Cirugia y Especialidades **18**, 156-170 [not seen].

GIBSON, H. J. (1935) A pathogenic diptheroid bacillus from a fatal case of meningitis. Jour. Path. and Bact. **41**, 239.

GIFFORD, REBECCA, and EVELETH, D. F. (1942) Listerellosis in a goat. Amer. Vet. Med. Assoc. Jour. **101**, 413-417.

GILL, D. A. (1931) "Circling disease" of sheep in New Zealand. Vet. Jour. **87**, 60-74.

———— (1933) Circling disease: a meningo-encephalitis of sheep in New Zealand. Notes on a new species of pathogenic organism. Vet. Jour. **89**, 258-270.

———— (1937) Ovine bacterial encephalitis (circling disease) and the bacterial genus *Listerella.* Austral. Vet. Jour. **13**, 46.

GOODPASTURE, E. W. (1924) Spontaneous encephalitis in rabbits. Jour. Infect. Dis. **34**, 428-432.

GRAHAM, ROBERT (1939) *Listerella* encephalitis or encephalomyelitis in domestic animals. Amer. Vet. Med. Assoc. Jour. **95**, 289-292.

———— DUNLAP, G. L., and BRANDLY, C. A. (1938) Ovine and bovine listerellosis in Illinois. Science **88**, 171-172.

———— ———— and LEVINE, N. D. (1940) Studies on *Listerella.* III. Experimental listerellosis in domestic animals. Cornell Vet. **30**, 268-290.

———— HESTER, H. R., and LEVINE, N. D. (1939) *Listerella* from a premature bovine fetus. Science **90**, 336-337.

———— ———— ———— (1940A) Studies on *Listerella.* I. A *Listerella* strain isolated from a premature bovine fetus. Jour. Infect. Dis. **66**, 91-96.

———— ———— ———— (1940B) Studies on *Listerella.* II. Field outbreaks of listerellosis in sheep and cattle. Cornell Vet. **30**, 97-111.

———— ———— ———— (1941) *Listeria* (*Listerella*) infections in domestic animals. Ohio State Vet. Med. Assoc. Proc., 58th Ann. Mtg.

———— and LEVINE, N. D. (1943) Studies on listerellosis. V. A note on the use of large doses of formalin-killed broth cultures in the experimental prophylaxis of ovine encephalitis. Amer. Vet. Med. Assoc. Jour. **102**, 365-367.

———— MORRILL, C. C., and LEVINE, N. D. (1940) Studies on *Listerella.* IV. Unsuccessful attempts at immunization with living and dead *Listerella* cultures. Cornell Vet. **30**, 291-298.

HARVEY, P. C., and FABER, J. E. (1941A) Some biochemical reactions of the *Listerella* group. Jour. Bact. **41**, 45-46.

———— ———— (1941B) Studies on the *Listerella* group. I. Biochemical and hemolytic reactions. Jour. Bact. **42**, 677-687.

HENDERSON, J. A. (1941) An outbreak of listeriosis in sheep. North Amer. Vet. **22**, 545-546.

HENRICSON, T. (1943) Ett fall av listerellos hos hare. Svensk Vet. Tidskr. 48, 1-9 [abstracted in Vet. Bul. **13**, 312].

HOFFMAN, H. A. (1941) Observations on a case of listerellosis in sheep. Amer. Vet. Med. Assoc. Jour. **98**, 234-235.

HURT, H. R., LEVINE, N. D., and GRAHAM, ROBERT (1941) Isolation of *Listeria* (*Listerella*) from the chicken. Amer. Jour. Vet. Res. **2**, 279-280.

HUTNER, S. H. (1942) Some growth requirements of *Erysipelothrix* and *Listerella.* Jour. Bact. **43**, 629-640.

JENSEN, G. W., and GAY, W. J. (1941) Ovine listerellosis. Report of an outbreak in a purebred flock. North Amer. Vet. **22**, 601-605.

JONES, F. S., and LITTLE, R. B. (1934) Sporadic encephalitis in cows. Arch. Path. **18**, 580-581.

JONES, T. C. (1940) The relation of brucellosis to periodic ophthalmia in horses. Amer. Jour. Vet. Res. **1**, 54-56.

JONES, T. C. (1942) Equine periodic ophthalmia. Amer. Jour. Vet. Res. **3**, 45-71.

JULIANELLE, L. A. (1940) The function of *Listerella* in infection. Internal Med. Ann. **14**, 608-620.

———— (1941A) Biological and immunological studies of *Listerella*. Jour. Bact. **42**, 367-383.

———— (1941B) The identification of *Erysipelothrix* and its relation to *Listerella*. Jour. Bact. **41**, 44-45.

———— (1941C) The identification of *Erysipelothrix* and its relation to *Listerella*. Jour. Bact. **42**, 385-394.

———— and MOORE, ELIZABETH (1942) Pathological changes in *Listerella* infection, particularly of the eye. Amer. Jour. Path. **18**, 813-825.

———— and PONS, C. A. (1939A) Identificatiout of *Listerella monocytogenes*. Soc. Exp. Biol. and Med. Proc. **40**, 362-363.

———— ———— (1939B) Immunological and serological reactions of *Listerella monocytogenes*. Soc. Exp. Biol. and Med. Proc. **40**, 364-365.

JUNGHERR, ERWIN (1937) Ovine encephalomyelitis associated with *Listerella* infection. Amer. Med. Assoc. Jour. **91**, 73-87.

KING, L. S. (1940) Primary encephalomyelitis in goats associated with *Listerella* infection. Amer. Jour. Path. **16**, 467-478.

LANG, F. J. (1928) Zur Monozytenfrage. Folia Haematol. **36**, 383-389.

LEVI, G. M., and PENATI, F. (1934) I fondamenti sperimentali e clinici nella valutazione della origine dei monociti. Arch. per le Sci. Med. **58**, 773-796.

———— ———— (1935) Ricerche sulla linfomonocitosi infettiva del coniglio (Infezione da bacillo monocitogene). II. Reperti istopatologici negli organi ematopoietici. Haematol. **16**, 317-343.

MATHEWS (1928) Encephalitis in calves. Amer. Vet. Med. Assoc. Jour. **73**, 513-516.

MORIN, L. N. (1938) Studies of a malady in sheep. Amer. Vet. Med. Assoc. Jour. **93**, 32.

MORRIS, M. C., and JULIANELLE, L. A. (1935) A study of an ocular infection induced experimentally with *Bacterium monocytogenes*. Amer. Jour. Ophthal. **18**, 535-541.

MURRAY, E. G. D., WEBB, R. A., and SWANN, M. B. R. (1926) A disease of rabbits characterized by a large mononuclear leucocytosis, caused by a hitherto undescribed bacillus *Bacterium monocytogenes* (n. sp.). Jour. Path. and Bact. **29**, 407-439.

MUTH, O. H., and MORRILL, D. R. (1942) An outbreak of listerellosis in Oregon sheep. Amer. Vet. Med. Assoc. Jour. **100**, 242-243.

NETTLESHIP, A. (1942) On infectious mononucleosis. Soc. Exp. Biol. and Med. Proc. **49**, 116-117.

NYFELDT, AAGE (1929) Etiologie de la mononucléose infectieuse. Soc. de Biol. [Paris] Compt. Rend. **101**, 590-592.

———— (1932) Klinische und experimentelle Untersuchungen über die Mononucleosis infectiosa. Folia Haematol. **47**, 1-144.

OLAFSON, PETER (1940) *Listerella* encephalitis (circling disease) of sheep, cattle, and goats. Cornell Vet. **30**, 141-150.

PALLASKE, G. (1940) Ueber das Vorkommen einer seuchenhaften Encephalomyelitis purulente bei Schafen in Deutschland (Listerella-Infektion). Berlin. Tierärztl. Wchnschr. (Sept. 13), 441-445 [abstracted in Vet. Bul. **12**, 571].

PATERSON, J. S. (1937) *Listerella* infection in fowls. Preliminary note on its occurrence in East Anglia. Vet. Rec. **49**, 1533-1534.
———— (1939A) The present position regarding *Listerella monocytogenes* infection in animals and man. Vet. Rec. **51**, 873-876.
———— (1939B) Flagellar antigens of organisms of the genus *Listerella.* Jour. Path. and Bact. **48**, 25-32.
———— (1941) Listerella-Infektion bei Hühnern in Deutschland. Berlin. Tierärztl. Wchnschr. (Sept. 12), 441-445 [abstracted in Vet. Bul. **12**, 571].
———— (1943) Weitere Untersuchungen über die Listerellainfecktion der Schafe. Ztschr. f. Infektionskrank., Parasitäre Krank. u. Hyg. der Haustiere **59**, 125-145 [abstracted in Vet. Bul. **13**, 312].
———— (1940A) The antigenic structure of organisms of the genus *Listerella.* Jour. Path. and Bact. **51**, 427-436.
———— (1940B) Experimental infection of the chick embryo with organisms of the genus *Listerella.* Jour. Path. and Bact. **51**, 437-440.
———— (1940C) A case of naturally occurring listerellosis in an adult rabbit. Jour. Path. and Bact. **51**, 441-442.
———— (1940D) Studies on organisms of the genus *Listerella.* IV. An outbreak of abortion associated with the recovery of *Listerella* from the aborted foetuses. Vet. Jour. **96**, 327-332.
———— (1941) Personal communication.
PENATI, F., and LEVI, G. M. (1935A) Ricerche sulla linfomonocitosi infettiva del coniglio (Infezione da bacillo monocitogene). I. Ricerche ematologiche. Haematol. **16**, 261-275.
———— ———— (1935B) Ricerche sulla linfomonocitosi infettiva del coniglio (Infezione da bacillo monocitogene). III. Origine degli elementi monocitari. Haematol. **16**, 409-419.
PIRIE, J. H. H. (1927) A new disease of Veld rodents, "Tiger River disease." So. Afr. Inst. Med. Res. Pubs. **3**, 163-186.
———— (1940) The genus *Listerella* Pirie. Science **91**, 383.
———— (1940) *Listeria:* change of name for the genus bacteria. Nature [London] **145**, 246 [abstracted in the Vet. Bul. **10**, 325].
POMEROY, B. S., FENSTERMACHER, REUEL, and ANDBERG, W. G. (1943) Listerellosis of sheep and cattle in Minnesota. Cornell Vet. **33**, 269-273.
PONS, C. A., and JULIANELLE, L. A. (1939) Isolation of *Listerella monocytogenes* from infectious mononucleosis. Soc. Exp. Biol. and Med. Proc. **40**, 360-361.
PORTER, J. R., and HALE, W. M. (1939) Effect of sulfanilamide and sulfapyridine on experimental infections with *Listerella* and *Erysipelothrix* in mice. Soc. Exp. Biol. and Med. Proc. **42**, 47-50.
———— and PELCZAR, M. J., JR. (1941) Some growth factor requirements of several strains of *Listerella monocytogenes.* Jour. Bact. **42**, 141.
PORZECANSKI, B., and DE BAYGORRIA, C. (1939) Arch. Soc. de Biol. Montevideo **9**, 98 [cited by Paterson, 1939B].
POSTON, M. A., UPCHURCH, S. E., and BOOTH, M. (1937) Jour. Ped. **11**, 515 [cited by Paterson, 1939B].
REZZESI, F. D. (1933) La infezione da "Bacterium monocytogenes" e il problema del monocito. I and II. Haematol. **14**, 239-258, 287-314.
SAVINO, E. (1940A) *Listerella monocytogenes* aislada de uni meningoencefalitis humana. Inst. Bact. [Argentina], Rev. **9**, 587-592 [not seen].
———— (1940B) Observación de listerelosis humana y acción terapéutica del "dagenan." Inst. Bact. [Argentina], Rev. **9**, 593-601 [not seen].
———— and VILLAZON, N. MORALES (1941) Estructura del anttigeno ciliar de *"Listerella monocytogenes."* Semana Med. **1**, 732-736 [not seen].

SCHMIDT, VIGGO, and NYFELDT, AAGE (1938) Ugeskr. f. Laegar. **100**, 336 [cited by Paterson, 1939B].

SCHOENING, H. W., and CREECH, G. T. (1933) An agglutination test for swine erysipelas. Amer. Vet. Med. Assoc. Jour. **82**, 503-508.

SCHULTZ, E. W., TERRY, M. C., BRICE, A. T., JR., and GEBHARDT, L. P. (1934) Bacteriological observations on a case of meningoencephalitis. Soc. Exp. Biol. and Med. Proc. **31**, 1021-1023.

——— ——— ——— ——— (1938) *Listerella monocytogenes:* a cause of meningo-encephalitis in man. Soc. Exp. Biol. and Med. Proc. **38**, 605-608.

SCHWARTE, L. H., and BIESTER, H. E. (1942) *Listerella* infection in cattle. Amer. Jour. Vet. Res. **3**, 165-176.

SEASTONE, C. V. (1935) Pathogenic organisms of the genus *Listerella.* Jour. Exp. Med. **62**, 203-212.

SLABOSPITS'KII, T. P. (1938) Pro noviĭ mikroorganizm, vidileniĭ vid porosyat. Nauk. Zap. Kiev. Vet. Inst. **1**, 39-49 [abstracted in Vet. Bul. **12**, 367].

TESDAL, M. (1934) Fatal meningitis caused by a *Corynebacterium.* Acta Med. Scand. **83**, 351-358 [cited by Julianelle, 1940].

WALLBACK, GÜNTER (1934) Sur la monocytose infectieuse du lapin par le bacille monocytogène. Arch. d'Anat. Micros. **30**, 275-294.

WEBB, R. A., and BARBER, MARY (1937) *Listerella* in human meningitis. Jour. Path. and Bact. **45**, 523-539.

WITTS, L. J., and WEBB, R. A. (1927) The monocytes of the rabbit in *B. monocytogenes* infection. A study of their staining reactions and histogenesis. Jour. Path. and Bact. **30**, 687-712.

WRIGHT, H. A., and MACGREGOR, A. R. (1939) A case of meningitis due to *Bacterium monocytogenes.* Jour. Path. and Bact. **48**, 470-472.